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STUDIES IN THE DEVELOPMENTAL ANATOMY OF PHLOX DRUMMONDII HOOK. III. THE APICES OF THE MATURE PLANT [1]

Helena A. Miller and Ralph H. Wetmore

In the two preceding parts of this study the ontogeny of the *Phlox* plant (*P. Drummondii* Hook.) was followed through the embryo and seedling stages. In the concluding part of this study, attention is directed to the developmental changes in those portions of the older plant in which there is a retention of meristematic activity, i.e., the vegetative and floral apices and the root apex.

Observations.—The *vegetative* apex of the shoot of *Phlox Drummondii* is composed of a central mass of seemingly haphazardly arranged cells, the "corpus," covered by a mantle of regularly arranged cells, usually one or two layers thick, the "tunica" (Schmidt, 1924). Evidence suggests (Miller and Wetmore, 1945a, 1945b) that, at the time of the first appearance of each foliar primordium on the epicotyledonary meristem, a continuous procambial strand can be traced to the new primordium from the vascular system below. Each leaf primordium elongates by meristematic activity at its apex; the single procambial strand is also extended behind this advancing tip.

The subsequent increase in length of the leaf primordia, the increase in width through the initiation of marginal meristems, and the concomitant thickening, especially of the basal part of the leaf, from an adaxial thickening meristem offer little for new comment.

The procambial strands of the leaves in *Phlox* are increased in size predominantly by periclinal divisions, whereas divisions in the surrounding tissue are both periclinal and anticlinal. In this study it was not possible to determine with certainty whether periclinal divisions in the cells immediately adjacent to the procambial strand contributed to the strand, or whether the strand increased only by divisions within itself. The lack of compressed cells around the strand suggests that the increase in the size of the strand was due to divisions in both regions.

The procambial branches of the main procambial strand of each leaf become apparent initially on the flanks of the strand in the middle layer. Subsequent development of these veins is centrifugal, continuity apparently never being interrupted. The smaller procambial branches of the network in the mature leaf seem to appear independently in isolated patches in the middle layer of the leaf and eventually establish continuity with the larger veins. The same sequence of events takes place in the development of branch veins in the cotyledons (fig. 1, 2).

[1] Received for publication July 2, 1945.

Like the procambium, the protophloem proceeds acropetally into the successive leaves of the stem apex as was shown to be the case in the first epicotyledonary leaves (Miller and Wetmore, 1945b). In its acropetal development, the first appearance of a protophloem cell in a foliar primordium becomes evident on the mid-abaxial, outer margin of the

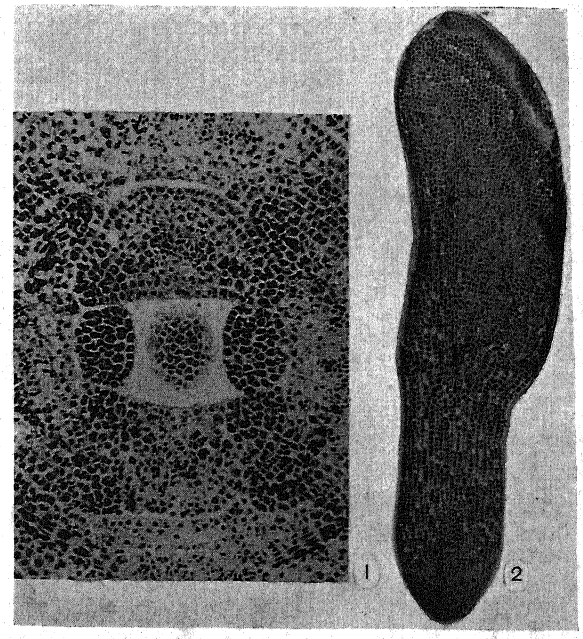


Fig. 1–2.—Fig. 1. Transverse section of a stem tip. ×113. —Fig. 2. Longitudinal section of an embryo showing the branching of the procambium in the cotyledon. ×45.

main procambial strand. Later-formed phloem elements appear both centripetally and on each side of the first-formed protophloem. The somewhat cylindrical strands at the tips of the leaves have more centripetal phloem development than lateral; the older, somewhat flattened, strand nearer the base of the leaves shows a greater lateral extension of phloem.

The first elements of protoxylem mature later than the protophloem sieve elements, appearing first at or near the base of the leaf, occasionally at independent levels (fig. 3, leaf 3, left). Further maturation of protoxylem is centrifugal in the procambial leaf strand.

The relatively massive epicotyledonary meristem produces leaves in successive pairs. As these leaves develop, older leaves are gradually separated from one another by continued over-all growth between

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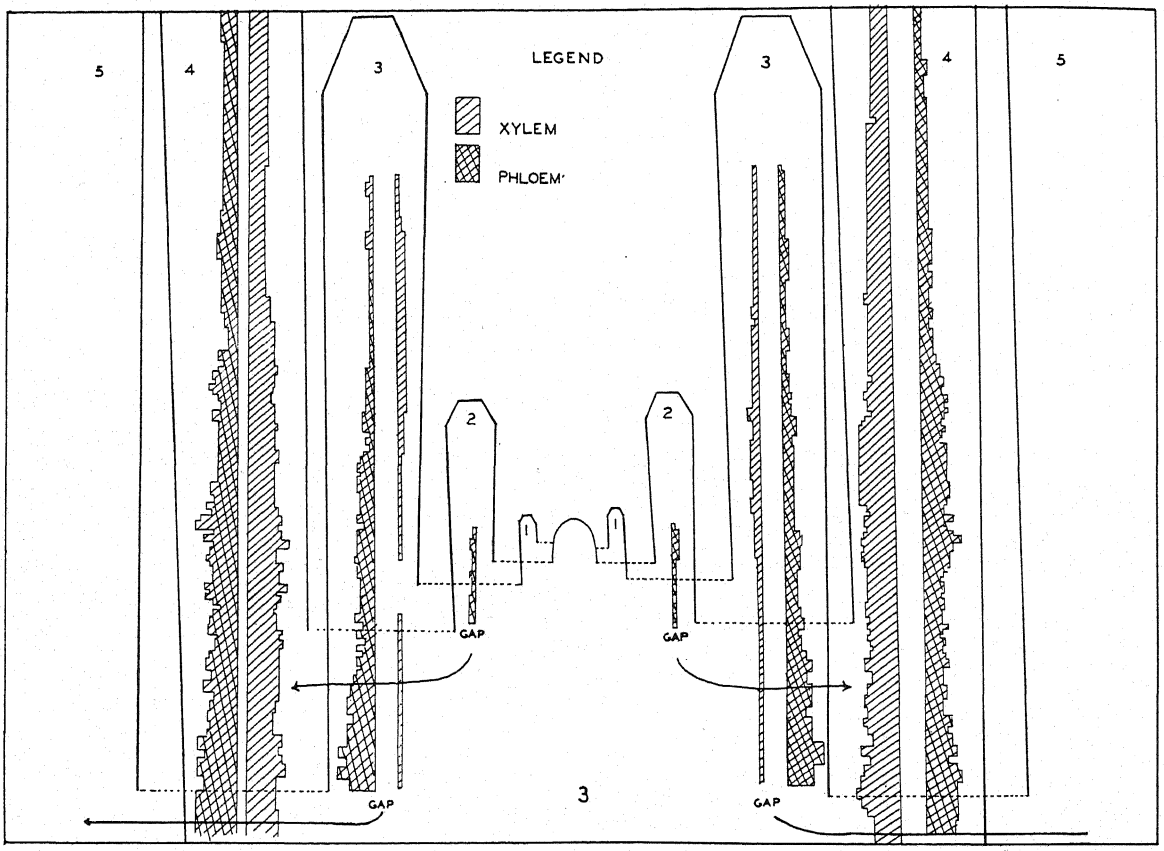


Fig. 3. Diagram of a *Phlox* stem apex, constructed from transverse sections, showing the relative amounts of matured xylem and phloem elements at various levels in the apex. The youngest pair of leaves is numbered (1), the next to the youngest (2), etc. Leaf pairs 2, 4, and 6 are decussate with leaf pairs 1, 3, and 5 on the plant, but are drawn in the same plane with them in the diagram to facilitate interpretation. Note the discontinuous xylem in leaf 3, left.

the leaf attachments or nodes, forming internodes. This internodal elongation results from an apparent correlated growth in the component zones of the axis. Growth results from anticlinal divisions and cell enlargements in the epidermal, cortical and pith regions, and from cell elongation in the vascular strands. Increased vacuolation accompanies the increase in size of cells in the epidermis, cortex and pith of the elongating stem. It also accompanies the enlargement of cells in each foliar primordium, appearing first on the abaxial, later on the adaxial side of a developing leaf.

After a second pair of epicotyledonary leaves is formed and carried upward by elongation, the second internode is formed. At this time a cotyledonary gap becomes evident with the gradual enlargement and vacuolation of a continuous zone of cells extending from the pith, along the cotyledonary leaf trace, across the cortical part of the stem and into the adaxial part of the cotyledons. Hence, the gap for a cotyledon does not appear until the procambium is present in the leaf above it.

A *vegetative bud* primordium, a small nest of darkly staining, isodiametric cells, is found in the axil of each leaf. This bud primordium appears lens-shaped in transverse section much as described by Louis (1935) for *Syringa*. Its cells show no tendency toward meristematic activity until three or four plastochrones later, that is, not until after the formation of the leaf gap at that node.

A study of transverse and longitudinal sections at the third node indicates that the surrounding cells become somewhat vacuolated, whereas those of the bud primordia remain densely staining and small, like those of the apical meristem. In these young buds, the primordium seems continuous below with the gap region, which at this time is comprised of denser, somewhat smaller, less vacuolated cells than are found on the surrounding regions. The authors believe that the young bud primordium has, from its beginning, a connection with that more centrally placed apical meristematic tissue which becomes the procambium of the adjacent node. It has not been possible to demonstrate this by any technique here employed—for the nodal conditions within the terminal bud, in which one finds the young axillary bud primordia, do not lend themselves to clear demarcation of regions or cell complexes.

A bud primordium in the axil of a leaf four nodes below the apex becomes a slightly elevated mound

as a result of meristematic activity (fig. 4, A–C, D). Just below the meristem, signs of central vacuolation (fig. 4 D) indicate the beginning of pith formation, leaving an annulus of darkly staining cells around it. At the level of the leaf buttress, the annulus becomes two crescentric arcs (fig. 4 A, BT; D). These two arcs at successively lower levels (fig. 4 C, BT) become confluent with the sides of the leaf gap (fig. 4 C, G, D). At this level the first pair of lateral branches to the leaf trace (LLT) are in continuity with the flanks of the main leaf trace (MLT) and also with the margins of the two bud traces (BT). At still lower levels, the flanking arc gets narrower radially (fig. 4 D) as the leaf trace assumes its place in the gap, the whole being eventually a part of the vascular cylinder of the stem. Cells in this crescentric bud trace have a greater length at levels progressively more distant from the bud apex. This evidence, from longitudinal sections, supports the conclusion that each crescentric bud trace is composed of an acropetally developing procambium. However, this does not necessarily establish the fact that the bud primordium has had continuous meristematic and procambial connection with the procambium of the stem.

In an older axillary bud possessing three pairs of leaves, the relationships are essentially as described for the younger bud (fig. 4 E–H). However, the individual leaf traces to the leaves of the bud can now be followed. The oldest pair of leaves (3 A) is at right angles to the leaf in the axil of which the bud is found. The trace to each of these leaves (3 A in G) occupies a median position in each crescentric arc. At the level at which each of these leaf traces "enters" its leaf, a small trace "separates" from each flank and is continuous upward around the gap of leaf 3, "uniting" above and eventually becoming a trace of 1 (fig. 4 E). On the flanks of each crescentric arc, another procambial strand (2A in G) can be seen. These strands at adjacent ends of the two crescentric procambial arcs form a single trace in the annulus of procambium (2A in F). The pair of traces so formed are the procambial supply to leaf pair 2 (E).

In the lower part of the vascular connection of the bud with the stem, marginal limits of each of these strands are not clearly distinct in sections because of the obliquity of the connections (fig. 4 H). Exact counts of mature protoxylem and protophloem elements are, therefore, difficult to make. However, there can be no doubt that in the traces of leaf 3 (fig. 4 E–G), the number of mature protoxylem cells is largest near the base of the bud and successively smaller nearer the tip. Protophloem elements, more difficult to identify, also appear to be progressively fewer in number at successive levels from the base. Furthermore, the traces of leaf pair 2 have recognizable mature vascular elements only at a lower level than the older leaf pair 3. No mature vascular elements appear in the traces of leaf pair 1.

Corresponding counts have been made on older buds. No exception was found to the general conclusion that the older bud leaves had more mature protoxylem and protophloem elements than the successively later appearing, and therefore younger, leaves. Moreover, these mature elements were more numerous near the base of the bud than at a higher level, although the older leaf traces of the bud possessed these elements at higher levels than the younger. The authors are convinced that in *Phlox* the differentiation of protoxylem and protophloem develops acropetally into the leaf traces of the bud primordia. Because of the difficulty in recognizing mature protophloem, it was not possible to determine whether protophloem or protoxylem was the first to differentiate in the traces of the bud leaves. In no case was there evidence found of an independent differentiation of protoxylem or protophloem near the apex of the bud.

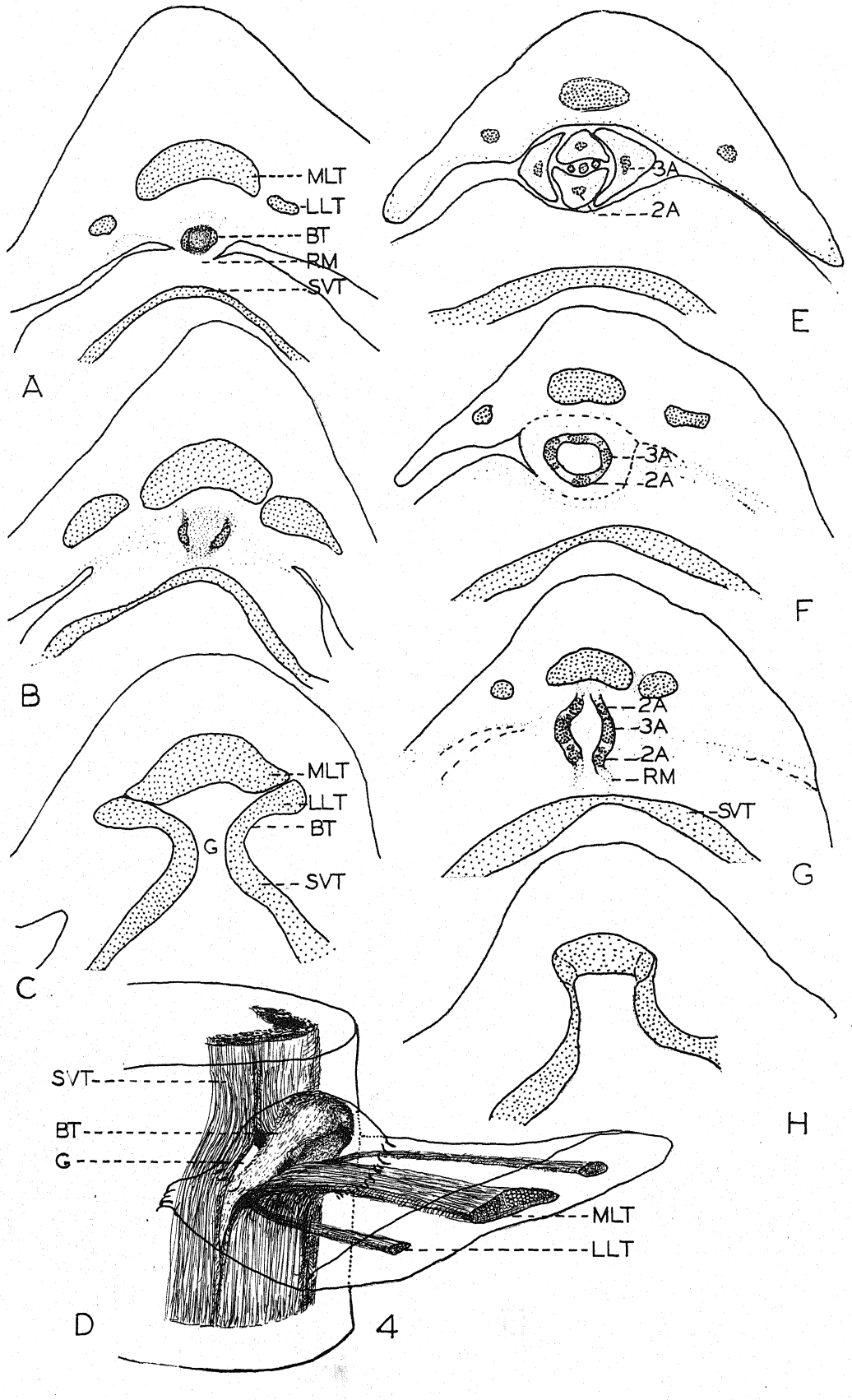
A vegetative bud may become a branch within a season and bear on its apex floral buds in the same way that the main axis does.

The floral shoot apex of *Phlox Drummondii,* when very young (fig. 5, 6), has a tunica composed usually of one or two layers of regularly arranged cells in which the divisions are almost entirely anticlinal, and, when older, of three to four layers (fig. 7, 8). These apices possess a relatively smaller corpus than the vegetative apices.

The phyllotaxis of a vegetative apex is decussate, but this phyllotaxis is lost when the floral apex is initiated. In a typical vegetative shoot, the buds appear in the axils of the third or fourth pair of leaves below the apex, but in the floral apex, buds appear soon after the foliar primordia are initiated, and may exceed the leaves, in the axils of which they appear (fig. 9, 10).

A study of the development in *floral buds* provides no striking contrast to the corresponding phenomena in vegetative buds. The decussate arrangement of vegetative leaves is replaced by a near-cyclic arrangement of floral parts. Floral buds develop faster, but soon become closed in their growth in that the apical meristem is entirely or almost entirely used in the formation of floral parts. Like the vegetative bud, the floral bud traces are still a pair of flanking connections between the margins of the gap and the trace of the subtending leaf, and these flanking connections become a pair of crescentric arcs higher up, and still higher, just below the apical meristem of the bud, they become an annulus. Procambial strands develop acropetally into these crescentric arcs and into the individual floral parts.

In the *development of the flower,* the primordia of the floral parts originate on the meristematic apex of the floral bud during the course of its development. The first to appear are the sepal primordia which have the appearance of leaf initials in longisection, but are unlike leaves in their arrangement, in that five primordia develop in succession, instead


MLT
LLT
BT
RM
SVT
A
3A
2A
E
3A
2A
F
B
MLT
LLT
BT
G
SVT
C
2A
3A
2A
RM
SVT
G
H
SVT
BT
G
MLT
LLT
D
4

of two opposite primordia initiated simultaneously at the node. A single procambial strand extends acropetally into each sepal, in the same way that the strands of procambium extend into the vegetative leaf primordia, and like the vegetative leaves, quickly acquire two branch veins. Centrad to the place of origin of the sepal primordia, five more initials, the stamen primordia, appear on an outer, circular zone of the remaining apical meristem. They elongate rapidly, and as they grow, a single procambial strand is extended acropetally into each. These primordia develop early into normal stamens. Centrad to the stamen primordia, the remaining meristem resembles a mound. Soon three more primordia, the carpels, develop on this mound. A single procambial strand, the *dorsal strand,* extends into each of these primordia and then forms a pair of lateral branches in the same way that a single median strand extends into a foliar primordium of *Phlox,* becomes its midvein, and gives rise to a pair of lateral branches. However, in the course of development, branch strands from adjacent carpels become confluent, thereby producing the three *ventral strands.*

Alternate with the sepals and between the young sepals and young stamens, five petal primordia appear and are elongated, predominantly by cell division, about the time that the carpels are enlarging. The single procambial strand is quickly separated from the confronting stamen strand and is extended acropetally into each petal primordium from the procambial complex out of which also the sepals and the stamens are supplied. These primordia eventually develop into petal lobes at the top of a corolla tube, which results from zonal growth (a ring of accelerated growth in the basal region) after the primordia are delimited.

The carpels are, meanwhile, increased in size, for a time almost entirely by cell division. Although these primordia are distinct when they are initiated, at a later stage they are free only at their tips. The localized areas of the floral apex which give rise to the initials of the carpels become enlarged in later development. Likewise, all of the meristem except three isolated spots immediately centrad to the points of initiation of the carpel primordia eventually becomes involved in the enlargement of the compound pistil (fig. 11). Thus originates a central column of cells which is connected in three regions with the lateral margins of the carpel primordia. The spaces left by the inactivity of the three isolated spots become the three locules. The structure resulting from this pattern of growth is a three-loculed ovary with a central axis bearing three placental ridges. Into each placental ridge a ventral strand develops acropetally. On each placental ridge appear one or two ovules, and within each ovule is formed a megasporocyte. Carpellary enlargement henceforth is caused by general cell division and elongation until the ovary is complete with three to six ovules, each containing a normal eight-nucleate embryo sac. Only one seed matures in each loculus. The style, very short in early stages of development, becomes long in later stages and is divided into three free lobes at the tip. On these free stigmas, hairs and secretory cells are developed.

Root apex.—The root apex of a mature plant is no different from the root apex of a seedling.

Discussion.—The interpretation of the developing vegetative and floral apices in angiosperms has been greatly facilitated by the comprehensive reviews of pertinent literature of Foster (1939, 1941, 1942), Esau (1943b) and Sifton (1944). Recent significant investigations by such workers as Esau (1942, 1943a, 1945), Majumdar (1942) and Engard (1944) have added valuable observations and discussions. By contrast, the interpretation of root apices at present is more difficult. As Foster (1942) has pointed out, a comparative study of the structure and developmental behavior of angiospermous root apices is necessary before we can have an understanding of the organization of roots. Furthermore, our interpretation of the origin of shoot and root apices would undoubtedly be greatly facilitated by an extension of our knowledge of angiospermous embryos. Unfortunately, the extensive reviews on the embryology of angiosperms by Schnarf (1929) and Souèges (1936, 1937) are still too limited to provide a proper background for an interpretation of the developing plant on a comparative scale. Certainly Johansen, in his recent review article (1945), has pointed out the need for work in this field. He states (p. 103), "It is impossible to comprehend how histogens arise, what their nature is, how the component cells are interrelated, and so on, unless the start is made in the embryo. . . . It is the writer's strong contention that the start must be made in the young embryo—at the very first histogenic cell. Otherwise, only an isolated phase of the entire story is told. . . . The histogen concept needs to be restated anew from an entirely different standpoint, that of the embryonic origin of the histogens."

The present study is an attempt to interpret the organization of a mature *Phlox* plant in the light of its embryological development. It has been pointed out (Miller and Wetmore, 1945a) that a pattern of development is already established in the young embryo before any elements are matured.

Fig. 4. A–C. Camera lucida drawings of transverse sections through a node showing a young vegetative bud. MLT—main leaf trace, LLT—lateral leaf trace, RM—residual meristem, BT—bud trace, SVT—stem vascular tissue, G—gap. ×40. D. Three-dimensional diagram of bud shown in sections A–C. E–H. Camera lucida drawings of transverse sections through a node showing an older bud with three pairs of leaves. B3A—leaf of the oldest pair of bud leaves, B2A—leaf from the next oldest pair of bud leaves. Other letters with the same meaning as above. ×40.

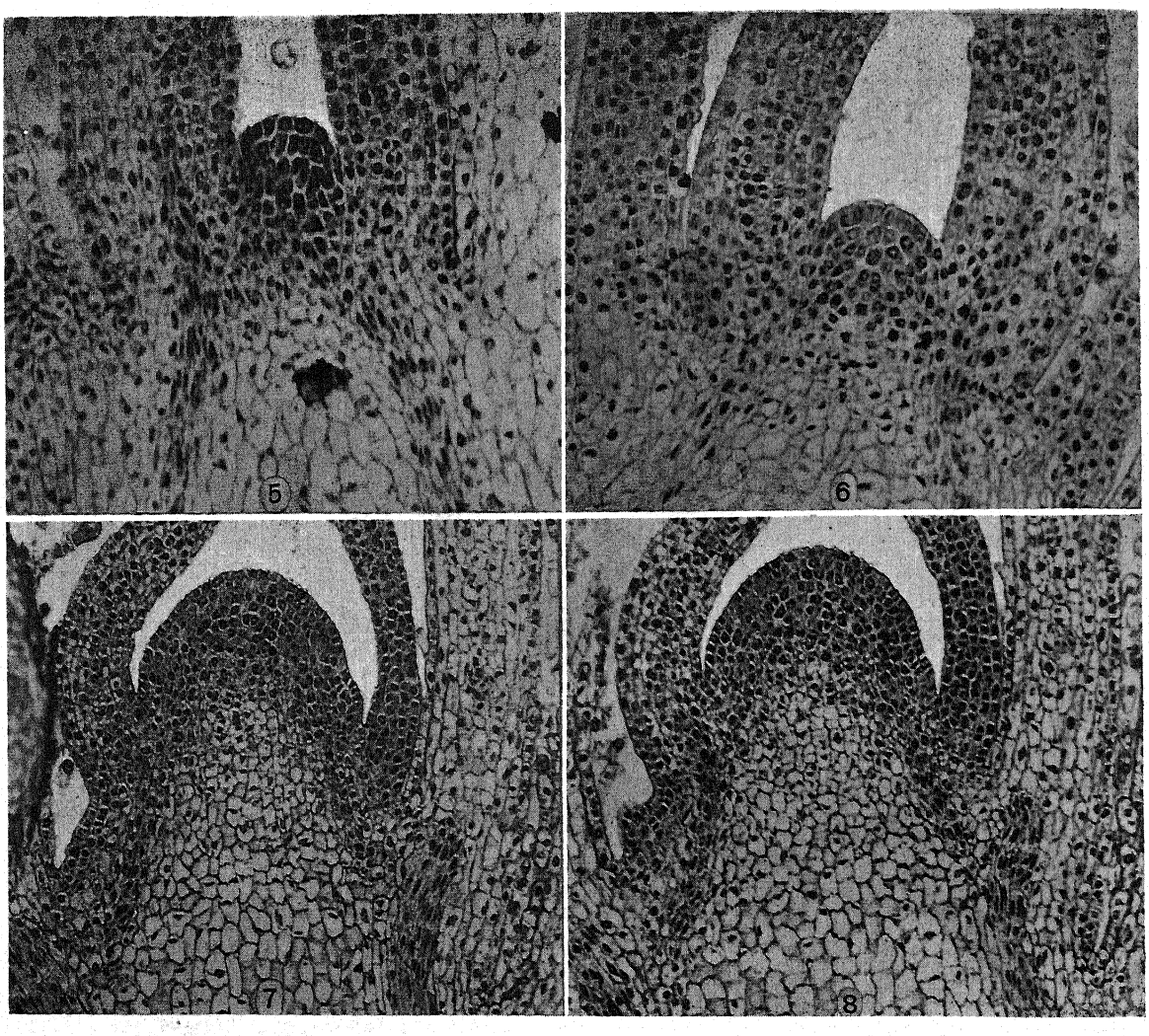


Fig. 5–8.—Fig. 5, 6. Longitudinal sections of young floral buds. Note the extension of the procambium into them. ×150.—Fig. 7, 8. Adjacent longitudinal serial sections, 10 μ thick, of a floral bud older than those in figures 5 and 6. Note the continuity of the procambial strand. ×170.

When mature elements do appear, they are formed within the established pattern. At no time, in consequence, can the plant be considered other than an entity; growth may involve extensions, but even these extensions are in keeping with this fundamental pattern. Thus with the advent of germination, a root is formed on the lower end of the plant by the activity of the already-formed root meristem. The pattern present in the lower hypocotyl of the embryo proceeds acropetally into this developing root. Shortly after the initial rapid increase of the root, following germination, the meristem between the cotyledons becomes extended upward, and in turn foliar primordia are initiated upon it. With the addition of the epicotyledonary shoot through the activity of this apical meristem, there is an extension of the pattern of the upper hypocotyl of the embryo into it. As the leaf primordia develop on the apex, part of the initial pattern is extended into them. Hence, the resulting pattern for the shoot is a modification of the original one in the upper embryo in that appendages develop resulting from more rapid activity in localized regions of the growing epicotyl.

Accompanying this increase in size and the extension of pattern, there is a maturation of vascular elements. It was noted that mature elements occurred in the cotyledons as early as the late embryo. The wave of maturation of more elements spreads upward into the cotyledons and downward into the hypocotyl, continuing in these directions after germination as the root is formed. This wave of maturation includes both protoxylem and protophloem.

When the wave of elongation in the seedling shifts from the root to the epicotyl, the maturation of elements follows. The *protophloem* which matures at a faster rate in the early advancing root is also first to mature in the leaf primordia. This wave of phloem maturation spreads from the region of mature elements in the upper hypocotyl acro-

petally into these leaf primordia. The *protoxylem* elements for the leaves, by contrast, begin to mature independently at their bases and the wave of maturation spreads from the leaf base acropetally in the foliar procambial strand to their tips, and basipetally from their bases to the region of mature xylem below.

It was seen that, in the developing embryo, the relation of xylem and phloem areas to each other in the existing pattern varies from an endarch, centrifugally developing xylem, and a centripetally developing phloem (initiated at the outer margin of the bundle)—in the cotyledonary and epicotyledonary leaf strands and in the upper cylinder of the hypocotyl—to an exarch, centripetally developing xylem alternating radially with a continuous, centripetally developing phloem—in the lower hypocotyl and root.

The transition region between the root and the shoot has received much attention and has been variously interpreted. From this study it appears that the transition region in *Phlox* arose after the proembryo stage, during the time when the cotyledonary primordia were developing and the procambium was being extended into them. It is already discernible in the torpedo stage, at a time when pith begins to be formed. It seems clear that an understanding of the so-called "inversion of bundles" in *Phlox* can be arrived at by a study of progressive stages of embryonic development. The interpretation rests on a study of the timing of the orderly sequence of events rather than on a descriptive interpretation of results. There is no "inversion of bundles" in *Phlox*.

As the shoot system develops, first vegetative buds and, later, floral buds appear as axillary nests of residual meristem which soon become activated. These buds are provided acropetally with procambium from the axis below. Also protoxylem and

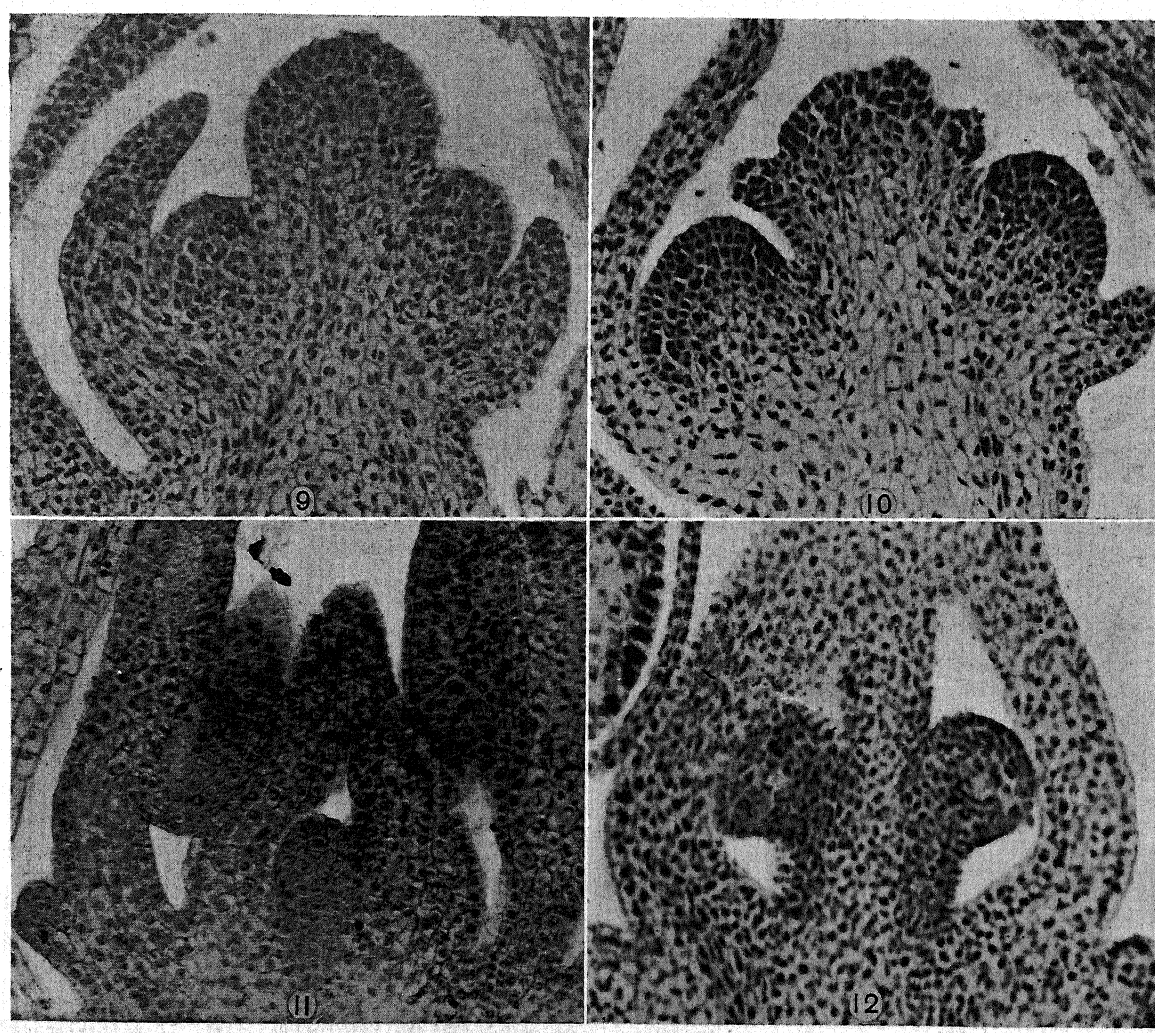


Fig. 9–12.—Fig. 9, 10. Longitudinal sections of successively older floral buds. ×203.—Fig. 11. Londitudinal section of a developing pistil, carpel primordia free.—Fig. 12. Longitudinal section of a developing ovary showing megasporocytes. ×203.

protophloem differentiate acropetally into these buds and into their appendages as well.

In this investigation of the orderly course of development in a *Phlox* plant, three issues merit further comment: (1) the comparative nature of the floral and vegetative apex; (2) the origin and vascularization of buds; and (3) the interpretation of tissue systems for the plant as a whole.

1. The structure and behavior of the floral apex are generally similar to those of the vegetative apex. Some variations exist: the floral apex is more rounded and has more rows of cells in the tunica than the vegetative. There is also a change in phyllotaxis from the decussate arrangement of the vegetative leaves to the cyclic pattern of the floral appendages. Both, however, have acropetal development of procambium. Esau (1943b) noted that an increasing number of reports have indicated an acropetal development of procambium into vegetative leaves. Engard (1944) in *Rubus* pointed out an acropetal development of procambium not only in vegetative but also in floral leaves, as have Satina and Blakeslee (1941) in *Datura*. This study on *Phlox*, therefore, offers one further example at variance with Grégoire's (1938) claim of basipetal development of procambium in vegetative leaves, although it supports his belief in the acropetal development into floral leaves.

2. As to the bud primordia, our study shows their origin in *Phlox* to be from residual axillary nests of cells, part of the original apical meristem. These bud primordia have never lost their connection with that part of the original apical meristem which becomes foliar procambium. This conclusion for *Phlox* is in accord with the reports of Louis (1935) for *Syringa, Arabis, Ligustrum, Urtica* and *Ranunculus*. However, it is in contrast to Koch's (1893) findings and to the more recent one of Majumdar (1942) for *Heracleum*. Both of these authors suggest that bud primordia originate from an aggregation of already vacuolated cells, and that only secondarily are these primordia connected with the axial cylinder. Few studies of the origin of buds have been reported. Since nodal conditions are so exceedingly diverse, a wide variety of structural types must be investigated before any generalizations are possible.

3. The problem of tissue systems for vascular plants is still controversial. Foster (1942) has considered the historical background, philosophical justification, and present status of Hanstein's (1868) histogens, Sachs' (1875) three-tissue systems, and the more elaborate twelve anatomico-physiological tissue systems of Haberlandt (1914). They need not be debated here in their general or their utilitarian aspects. It should be pointed out, however, that these three classical systems for interpreting the organization of plants were elaborated for shoots and roots, and not for embryos from which the organization originates. Hanstein (1870) did extend his histogens to embryos, but, as Johansen (1945, p. 92) points out, subsequent workers in angiospermous embryology have not continued to use the histogens as Hanstein applied them.

A further difficulty faces the student in embryology. In the proembryo, generalized meristematic activity prevails; later this activity becomes gradually localized in the apical regions, the remaining parts of the plant being of varying stages of maturation. Lacking exact criteria by which one can recognize mature plant cells, it becomes difficult to know when one should apply formative terms to these layers, zones or tissues, or when one should consider them mature.

To the authors of this paper these difficulties seem easily solved. They suggest that the three simple tissue systems of Sachs (1875) are adequate for the recognition of zones or layers in any or all parts of the primary body of the plant at any and all times after they have been initiated. Essentially Sachs considered these three systems as topographically, rather than histologically or physiologically distinct:

a. An epidermis or epidermal system—a peripheral layer covering embryos, and the young parts of roots and shoots, other than the apical meristems. This layer becomes a boundary layer between the plant and its external environment. It can be recognized as a distinct layer from its inception in the embryo, and, unless destroyed by secondary activity, it is continuous, except over the meristematic apical regions of root, leaf, stem and bud. Its cells may divide when young, or at times even later. These divisions are usually anticlinal, thus perpetuating and extending the layer.

b. A vascular system of procambium or of mature or maturing cells. It may be a solid core (protostele), a pith-containing cylinder (siphonostele), an anastomosing system of strands in a cylinder (eustele), or a system of scattered strands (atactostele).

c. A ground tissue system, comprising that tissue of a plant or of an organ which still remains after the formation and development of the epidermal tissue and the vascular system. In stems, this system consists of a *cortex* and a *pith* (usually present in the vascular plants), in roots a *cortex* and rarely a *pith* in leaves a *mesophyll*. Cortex, pith and mesophyll are often not clearly demarcated from the vascular system; all have diversified cell types at later stages of development of the plant.

The authors recognize that their suggestion is not an answer to all problems. If, however, it does permit the recognition of zones throughout the life of a vascular plant, it supplies a need. Moreover, one can still employ the term *protoderm* for the young epidermis, *ground meristem* for the young cortex, and *procambium* for developmental stages of the vascular system. There is significance in the fact that the apical meristems gradually merge into the developmental stages of the tissue systems, and that these developmental stages gradually take on the form of the various expressions of the mature

epidermis, the cortex or the primary vascular system. The important fact remains that the three tissue systems are continuously and topographically recognizable at whatever stage, and for as long as each system is an intact part of the primary body.

SUMMARY

The vegetative shoot apex in *Phlox Drummondii* possesses a tunica of one to two layers while the floral apex has three or four layers. The foliar appendages are formed in decussate pairs on the shoot apex of a seedling or a mature shoot and are extended by cell division and cell enlargement. Vacuolation in each foliar primordium occurs first on the abaxial side of the procambial strand and later on the adaxial side.

A procambial strand develops acripetally into each foliar primordium from the flanks of that procambial strand which already has developed into the leaf immediately below.

Maturation of xylem and phloem elements appearing in the procambial strands follows the same pattern for the leaves of the vegetative apex as was present in the first pair of leaves. The protophloem matures acropetally from a region of mature phloem below. The first protoxylem elements mature after the phloem and at an independent position in the procambial strand of the leaf base, establishing connection with that below by basipetal maturation. Additional xylem elements in the strand mature in centrifugal direction, whereas additional phloem elements mature in a centripetal direction.

The locus of greatest numbers of matured elements of either xylem or phloem in a bundle seems to indicate both the center of maturation and the locus of initial maturation in the vegetative apices. In the seedling (Part II), however, the locus of greatest numbers of matured elements is not identical with the point of first maturation.

A leaf gap for a cotyledon and any succeeding leaf begins to be evident when the procambium of the leaf directly above is detectable.

Vegetative buds are formed by meristematic activity in axillary groups of cells at three or four nodes below the shoot apex. Within the bud meristem, procambial connections develop acropetally from the lateral margins of the leaf and stem vascular tissue at the node. Enlargement and vacuolation of cells cause the formation of cortex and pith of the protruding bud.

The loss of a decussate phyllotaxis is the first indication of the change from a vegetative to a floral apex. This is accompanied by the prompt appearance and excessive growth of the axillary buds at the leaf nodes while the leaves are still primordia. Procambium extends acropetally into these floral buds forming a cylinder. On the apex of the floral bud, sepals, stamens, carpels and petals develop in the order named. Procambium is extended acropetally into each floral appendage.

The development of the anatomy of the mature apices is a continuation of the beginnings of development which were evident in the early embryo. An interpretation of mature structures is facilitated by the study of successively older stages from the fertilized egg.

A topographical interpretation is suggested for the primary tissue systems of the vascular plants.

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CRITICAL NOTES ON THE ORIGIN AND DOMESTICATION OF THE CULTIVATED SPECIES OF CUCURBITA [1]

Thomas W. Whitaker and George F. Carter

INTEREST IN the problem of the origin and subsequent domestication of the three annual, cultivated species of *Cucurbita* (*C. pepo* Linn., *C. moschata* Poir., and *C. maxima* Duch.), has given rise to a large literature. Although no satisfactory theory for their origin has yet been proposed, it is quite generally agreed that all three species are endemic to the Americas. In a recent paper, L. H. Bailey (1943) makes the statement that, "One of the major problems before students of plants is to determine the origins of the three great species or groups of pumpkins or squashes, *Cucurbita pepo, C. maxima,* and *C. moschata,* all of which are supposed from historical and archeological evidences to be American."

The present report is a contribution toward the solution of some of these problems. On the basis of fresh data obtained during the course of a study of the distribution of the major crop plants of the Indians of the Southwest, we propose (a) to point out the essential differences between the three domesticated species; (b) to furnish evidence that there were at least two independent domestications of *Cucurbita pepo;* (c) to show that these separate domestications produced distinct varietal types; (d) to correlate these findings with previous work so as to construct a theoretical picture of the relationships of the entities which exist within the domesticated cucurbits.

Before entering into a discussion of the evidence which supports the points listed above, it is important to have a clear picture of the present distribution and archeological history of the group. The extensive and thorough work of the members of the Soviet botanical exploring expeditions has done much to clear up our ideas regarding the distribution of the domesticated cucurbits (Bukasov, 1930). They found *Cucurbita moschata* to be widely cultivated in Mexico, Guatemala, Panama, and Colombia, and a great many forms of *C. pepo* were found also in the northern portion of Mexico. According to Bukasov, *C. maxima* is found only in the southern portions of Peru and Bolivia, and in northern Chile.

The archeological evidence, obtained and recorded by Erwin (1931), establishes quite definitely the pre-Columbian use in North America of *Cucurbita pepo* and *C. moschata,* from which it is concluded that these two species are indigenous to North America. Vestal (1938) has been able to confirm this finding for *C. moschata.* He has identified as belonging to *C. moschata* a carbonized peduncle found in a pre-Columbian mound in Guatemala. The junior author has identified seeds from Peruvian urns that included *C. maxima* (Carter, 1945b). Several varieties were present in the urns. They seemed to be most closely related to our modern commercial Hubbard squash. There are other incidental bits of evidence that substantiate the points mentioned above. Thus the obvious conclusion may be drawn that all three species are of American origin, and that they were domesticated in pre-Columbian times.

ESSENTIAL DIFFERENCES BETWEEN CUCURBITA PEPO, C. MOSCHATA AND C. MAXIMA.—All three species have twenty pairs of chromosomes (McKay, 1931; Ruttle, 1931; Whitaker, 1930), and the extensive work of Erwin and Haber (1929) indicates that fertile crosses cannot be obtained from reciprocal matings of *Cucurbita maxima* with either *C. pepo* or *C. moschata.* On the other hand, these investigators were able to maintain hybrids from a cross of *C. pepo* by *C. moschata* through three generations. In this case the reciprocal mating was sterile. However, it is concluded from these investigations that crossing between *C. moschata* and *C. pepo* rarely, if ever, occurs in nature, although they are often planted together and such crossing could easily take place if the species were cross-fertile to any appreciable extent. The writers can verify this conclusion from their own experience in growing these plants.

The species under discussion are not easily distinguished from each other by any one morphological character. There are possibly five or six features that have diagnostic value, and a consideration of all of them usually permits one to arrive at a reliable decision regarding the identity of the specimen. The seeds of each species show differentiating characters which are probably the most easily applied and reliable means of separating them, but even here there are borderline cases that

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TABLE 1. *Essential morphological differences between the three cultivated species of Cucurbita.*

	C. pepo	*C. moschata*	*C. maxima*
1. Seed color	Tan	Grayish white to tan	White, or brown to bronze
Margin	Similar in color and texture to body of the seed	Deeper color than body of the seed; wavy or crinkly	Same color and texture as body of the seed
Scar	Horizontal or rounded	Horizontal or rounded	Slanting
2. Calyx-lobes	Awl-shaped, linear	Flattish, and in many varieties foliaceous or tending to be foliaceous	Narrow, linear, thin, filiform, long
3. Peduncles	Five-sided, grooved, not conspicuously flaring at attachment to fruit	Five-sided, grooved, distinctly flaring at fruit attachment	Roughly cylindrical, soft-spongy
4. Leaves	Blades strongly lobed with deep sinuses between lobes; harsh, rough, setose-prickly	Lobes pointed, sinuses absent; white spots at intersection of the veins; soft-hairy, without prickly setae	Roughly kidney-shaped, rounded in outline; obscurely lobed, or not lobed; not harsh or prickly; setae interspersed with soft hairs

require substantiating evidence from vine, flowers, and fruit as we shall explain later in this report. The greatest difficulty in identification involves the two species *C. pepo* and *C. moschata*. Both are fairly easy to distinguish from *C. maxima,* but they are not readily separated from each other unless there is available sufficient material of the key parts of the plants for study.

In table 1 we have listed the morphological characters which are most important in separating the three species.

There are certain exceptions to the distinctions made in table 1 which in the past have caused difficulty when identification was attempted from fragmentary and incomplete material.

1. Seeds of the Southwestern types of *Cucurbita pepo* have a faintly crinkly margin which in some forms closely approaches the distinctly crinkly or wavy margin of certain varieties of *Cucurbita moschata*. The calyx-lobes of these forms tend to be more plump and fleshy than those of typical *C. pepo,* again simulating the condition found in some varieties of *C. moschata*.

2. Although the typical peduncle of *Cucurbita moschata* is five-sided, grooved, and distinctly enlarged at the point of attachment to the fruit, there are a few atypical varieties in which the peduncle is not sharply angled but is roughly cylindrical, and somewhat spongy. (See Erwin, 1936, for discussion of aberrant forms of *C. moschata*.)

3. In the Southwestern pepos the leaves are not strongly lobed and the pronounced sinuses characteristic of the Eastern pepos are absent. Thus the leaves are roughly similar to those of *Cucurbita moschata*. White spots at the intersections of the veins are characteristic of the leaf blades of varieties of *C. moschata,* although in some of them they may be obscure or somewhat diffuse. White spotting is occasionally found in varieties of *C. pepo,* but in the latter species the spotting is less pronounced and is not necessarily associated with vein pattern.

MULTIPLE DOMESTICATION WITHIN CUCURBITA PEPO.—The evidence for the independent domestication of different groups of *Cucurbita pepo* has been treated in detail by Carter (1945a). Briefly this hypothesis is supported by the following facts. First, the archeological evidence indicates that a variety of *C. pepo* entered the southwestern part of the Puebloan area about 1000 A.D. The Puebloan cucurbit for the preceding 800 years had been *C. moschata*. The entry of *C. pepo* into the Pueblo area is associated with an expansion of the Hohokam culture of the Gila and Salt River Valleys and is accompanied by other unique Hohokam crops (*Gossypium hopi, Phaseolus acutifolius,* and a unique race of corn). The entry of *C. pepo* into the Southwest at this time and place is, therefore, to be correlated with the Hohokam culture. The origins of the Hohokam culture are obscure but agriculturally this culture is clearly not related to the eastern United States for it is equipped with crops of western Mexican origin which never reached the eastern United States in pre-Columbian times. Second, in the eastern United States *C. pepo* appears in the earliest agricultural horizons and is accompanied by a unique assemblage of locally domesticated plants (*Iva, Chenopodium, Ambrosia, Helianthus*) totally unrelated to the Hohokam assemblage. Although the eastern United States later obtained Mexican crops, they were the crops of Middle America and southern Mexico, rather than those of northwestern Mexico. Third, the botanical evidence, as presented herein, clearly indicates a marked divergence in the forms of *C. pepo* in the two areas.

In both the East and in the Gila-Colorado or northwestern Mexican area, then, there is evidence of an independent center of plant domestication. Significantly divergent forms of *Cucurbita pepo* are found to be the only domestic plants in common. The suggestion that they were independently domesticated is, therefore, advanced.

This suggestion is supported by the lack of evidence of contact between the two areas. As pointed out, the agricultural beginnings of these areas were separate. When these regions later obtained corn, they secured differing races, indicating differing

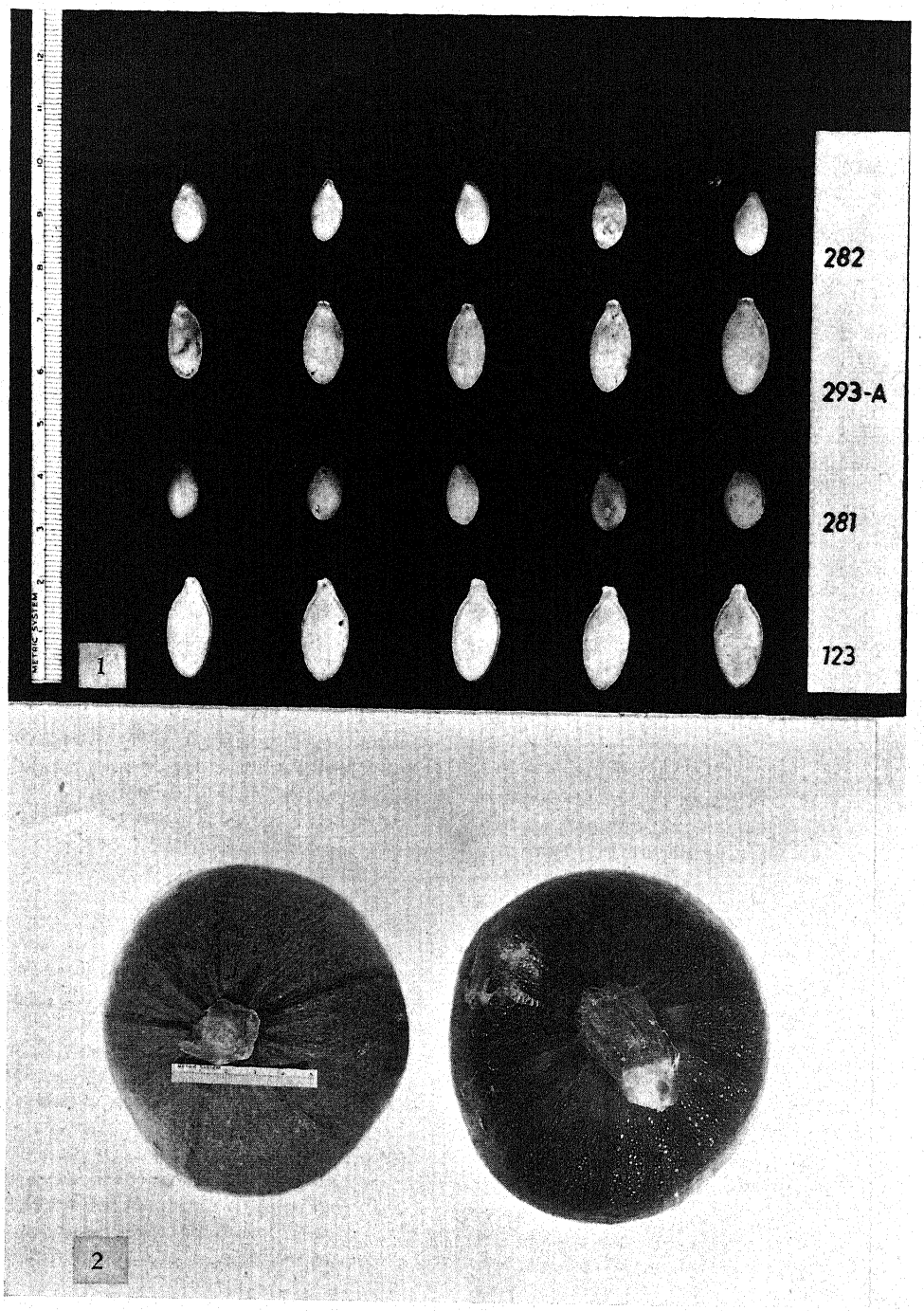


Fig. 1–2.—Fig. 1. Seed of four varieties of *Cucurbita pepo;* Golden Crookneck (#282); Black Zucchini (#293-A); Table Queen (#281); Southwestern *C. pepo* (#123). Note the larger, more elongated seed of the Southwestern domesticate as compared with the other varieties.—Fig. 2. Typical fruit of the southwestern *C. pepo.*

sources of supply. The key plants, aside from *Cucurbita pepo,* are reciprocally absent. Neither cotton nor the tepary bean spread to the East. The sunflower did not spread to the Gila-Colorado area, although it is present in the Puebloan area where other crops clearly derived from the East are found. This difference between the two areas extends through other, although presumably later, cultural features. To derive the *C. pepo* of the two areas from one area poses the question as to why such useful plants as the sunflower were rejected if the movement was from the east, or why cotton and the tepary bean were rejected if the movement was from the west. Hence we not only lack evidence

TABLE 2. *Differences between Eastern and Southwestern types of Cucurbita pepo.*

	Eastern types	*Southwestern types*
Vine type	Running or bush	Running
Leaf	Strongly lobed, deep sinuses	Slightly lobed, shallow sinuses
Seed	Mostly small, plump, white to tan	Long, flat, deep tan
Calyx-lobes	Awl-shaped, linear	Tendency to be flattish
Fruit	Scallops, pattypans, marrows, warted crooknecks	Smooth, slightly elongated, grayish skin color

of contact between the two areas at any time level, but we have strong circumstantial evidence indicating that there was no such contact.

If we concede that *Cucurbita pepo* was domesticated independently at least twice, we are immediately confronted with the problem of its origin. The facts at hand are not sufficiently critical to furnish a satisfactory answer to this puzzling question. However, it should be mentioned that wild progenitors exist in each area which may have formed the matrix from which domestication took place. *Cucurbita texana* A. Gray of central and southern Texas is certainly very closely related to *C. pepo,* and for some time it was generally regarded as an escaped form of *C. pepo* var. *ovifera.*

Recently this species has been examined in the field by several students of this group and they are firmly convinced that *Cucurbita texana* has every outward appearance of an indigenous plant rather than an escape (see Bailey, 1943). Thus there is no critical evidence that would eliminate *C. texana* as a possible primitive ancestor of the cultivated pepos.

In southwestern United States and northern Mexico there are perhaps three native species that would seem to offer potentialities for domestication. These three species, *Cucurbita palmata* S. Watson, *C. foetidissima* H. B. K., and *C. digitata* A. Gray, differ markedly in one important respect from *C. pepo.* All three are perennial forms with a fleshy, more or less fusiform, persistent tap root, as contrasted with the fibrous root system and annual habit of *C. pepo.*

DESCRIPTION OF A SOUTHWESTERN DOMESTICATE OF CUCURBITA PEPO.—The junior author has obtained evidence (Carter, 1945a) that a variety of *Cucurbita pepo* was cultivated by the peoples of the Gila-Colorado area from Pueblo II times, about 1000 A.D. During the course of field work, seed was discovered among the present-day descendants of these peoples that presumably is of the same type as that originally grown during the Pueblo II period. A comparison of the morphological characters of the vine, flower, fruit, and seed of this form shows that it is quite distinct from the common domesticated forms of *C. pepo* (fig. 1, 2) which have generally been considered to have reached their greatest diversity, and to have been domesticated, along the Atlantic Coast of eastern North America. Pollen mother cell counts indicate that this form has twenty pairs of chromosomes. There is no reason to think that it would not cross with other varieties of *C. pepo* and produce fertile progeny. Although the differences between the Eastern and the Southwestern domesticated forms are not outstanding qualitatively, the total pattern gives to the plants of the Southwestern domesticate a distinct varietal diversity. Differences between these types are tabulated in table 2.

In considering the differences recorded in table 2 between the Eastern types and Southwestern types of *Cucurbita pepo,* one point is worthy of attention. The deviations of the Southwestern types from their Eastern counterparts are mostly in the direction of *Cucurbita moschata.* The slightly lobed leaves with shallow sinuses, the tendency of the calyx-lobes to be broad and flattish, and the slightly wrinkled margin of the seed are all characteristics which, while found to a minor degree in the Southwestern *C. pepo,* are more pronounced and better developed in *C. moschata.* At the same time the characteristics mentioned above are absent for the most part in the Eastern domesticated varieties of *C. pepo.*

These observations suggest that there may have been some contamination of the Southwestern pepo, during the course of its development, with *Cucurbita moschata* genes. There is evidence that a transfer of genes between the two species could occur in spite of the fact that crossing between them rarely, if ever, takes place without the aid of artificial pollination. Erwin and Haber (1929) report fertile crosses between *C. pepo* and *C. moschata* when *C. pepo* was used as the female parent. No fertile seed was obtained from the reciprocal cross. This work agrees with results obtained by L. H. Bailey in some experiments carried out prior to 1900 and reported by him in summarized form in 1929 (see Bailey, 1929).

RELATIONSHIPS OF THE DOMESTICATED CUCURBITS.—We can best summarize our re-examination of the relationships of the domesticated cucurbits by reference to figure 3. This figure illustrates in diagrammatic fashion the presumed origin and relationships of the various species that have been concerned in the developmental history of the three species of domesticated cucurbits. It is based upon the information found in the work of Bailey (1943), Bukasov (1930), and Zhitineva (1930), and the observations we have made during the course of

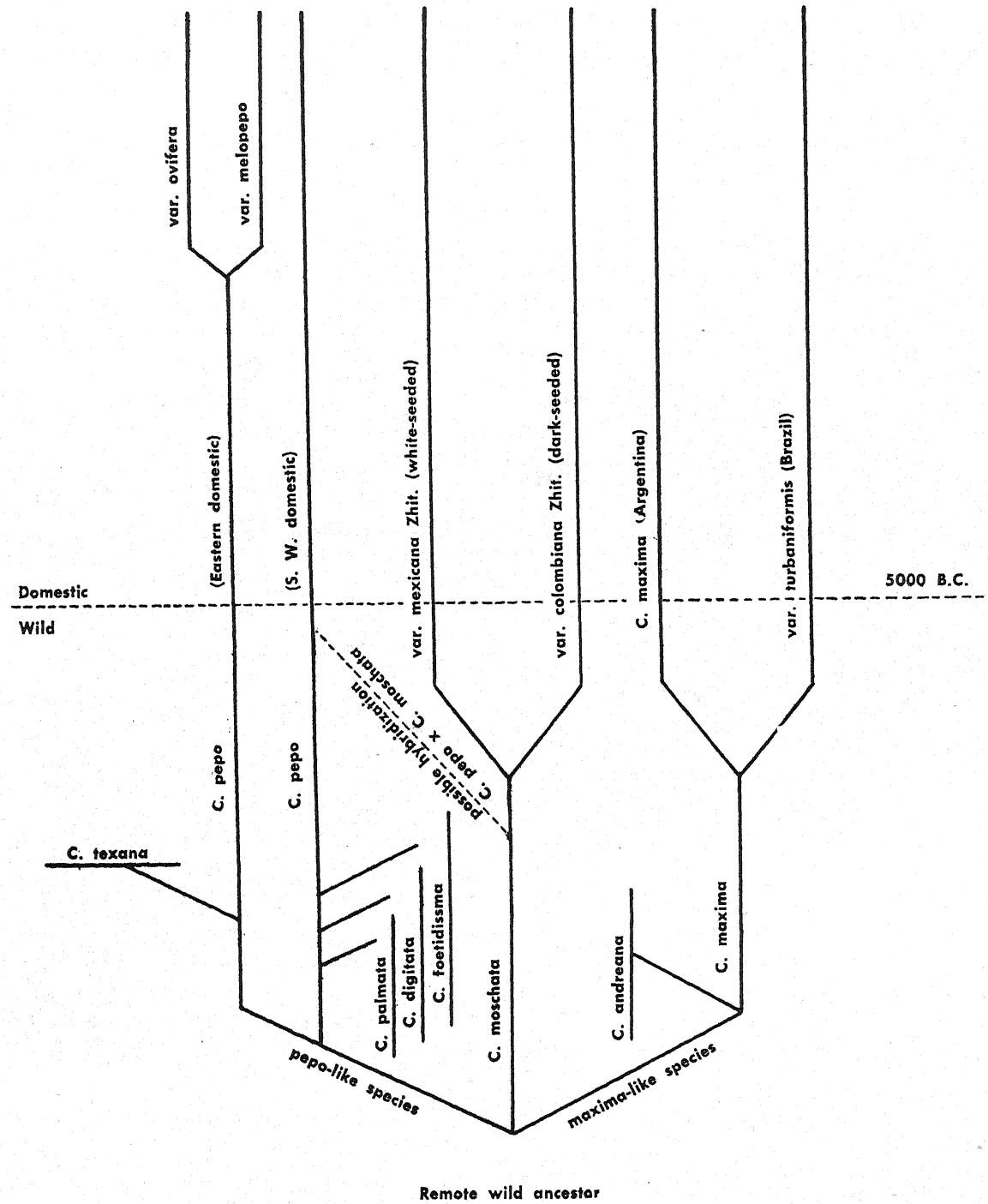


Fig. 3. Diagram illustrating the relationships of the three domesticated species of *Cucurbita*.

this study. The diagram is largely self-explanatory, but certain features may be worth noting.

The date 5000 B.C. is only a rough approximation, since it is obvious that domestication did not take place in all regions simultaneously. The place of most ancient occurrence is indicated either by the varietal names or by mention of the geographic area. Thus, according to Zhitineva (1930), the white-seeded forms of *C. moschata* are found only in Guatemala and southern and central Mexico, while the dark-seeded form is confined to Colombia.

One important point remains to be discussed. Sauer (1936), through a consideration of the distribution of the basic starch plants, concludes that there were several centers of domestication in the New World. This leads us to what we are convinced

is a principle of very wide application in plant species, namely, that in the various forms of a domesticated species we may actually be observing material that has been domesticated at different time levels and in different localities. Multiple domestication may account, in part, for the considerable genetic diversity found in domesticated species. Botanists should keep this point in mind when exploring some of the puzzling problems connected with the origin of domesticated plants.

SUMMARY

The distinguishing features of the three cultivated species of *Cucurbita* have been discussed, and practical criteria for their separation are suggested. The evidence for multiple domestication of *Cucurbita pepo* has been briefly reviewed with the purpose of showing that at least two of these domestications have resulted in distinct varietal types. A domesticated form of *C. pepo* from the southwestern United States is described for the first time and is shown to be quite distinct from the Eastern domesticates of this species from the Atlantic Coast region. These observations have been combined with those of previous investigators to produce a theoretical picture of the origin and relationships of the three cultivated species of *Cucurbita*.

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NEUROSPORA. III. BIOSYNTHESIS OF THIAMIN [1]

E. L. Tatum and T. T. Bell

INVESTIGATIONS OF X-ray and ultraviolet-induced biochemical mutant strains of *Neurospora* (Beadle and Tatum, 1941, 1945) have led to a clearer knowledge of the mechanism of biosynthesis of a number of biologically important compounds. In the case of tryptophane (Tatum *et al.*, 1944) and arginine (Srb and Horowitz, 1944), it has been possible to determine the particular reaction in the biosynthesis which fails as the result of a given gene mutation. The present investigation was undertaken with the aim of identifying in a similar fashion the biochemical reactions which normally lead to the synthesis of thiamin, but which cannot take place in the various thiamin-requiring mutant strains of *Neurospora* available for investigation (Beadle and Tatum, 1941, 1945; Tatum and Beadle, 1945).

EXPERIMENTAL.—Four thiamin-requiring mutant strains of *Neurospora* have been used in this investigation. Three of these, 9185, 18558, and 17084, were derived from *N. crassa* and one, 1090, from *N. sitophila* (Beadle and Tatum, 1945). Genetic studies (table 1), in which crosses with wild type were made and ascospores isolated in order, have indicated that each of these strains differs from normal by a single

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A part of the data reported here is to be included in a thesis submitted by the junior author to the School of Biological Sciences, Stanford University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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TABLE 1. *Segregation of thiamin mutant types in crosses with wild type.*

Strain number	Number of asci dissected	Number of asci showing second division segregations	Indicated distance from centromere
9185[a]	45	21	23
18558	24	4	8.3
17084	8	0	0
1090[b]	33	23	35

[a] Partly from data of G. W. Beadle and A. M. Srb.
[b] From data of G. W. Beadle.

gene. Each would, therefore, be expected to differ from normal in only one biochemical reaction involved in the normal biosynthesis of thiamin in *Neurospora*. Genetic tests have shown that the mutant genes in the three strains of *N. crassa* are not allelic, since wild-type spores were obtained from representative crosses. The non-allelic nature of the mutant genes in the available strains of *N. crassa* was substantiated by the formation of heterocaryotic cultures (Beadle and Coonradt, 1944) in liquid medium. Table 2 shows that almost normal growth was

TABLE 2. *Heterocaryon formation with N. crassa mutant strains 9185, 18558, and 17084.*

Strains	Growth mg. dry wt. in 6 days
17084	5.0
9185	1.5
18558	2.5
17084 + 9185	62.0
17084 + 18558	96.0
18558 + 9185	110.0

obtained when any two strains of *N. crassa* were inoculated into minimal medium. All strains used in these experiments were of sex *a*. The growth in these cultures is good evidence of heterocaryon formation since the mixture of any one mutant strain of *N. crassa* with *N. sitophila* 1090 did not give increased growth. In these cases interspecific heterocaryon formation did not take place nor was any symbiotic stimulation evident.

Growth of the mutant strains was followed in liquid medium (Horowitz and Beadle, 1943) using 20 ml. of medium in 125 ml. flasks. After three days incubation at 25°C. the mycelium was harvested, washed, dried at 100°C. and weighed.

The growth responses of the four strains to thiamin are given in figure 1. Mutant strains 9185 and 18558 show very similar responses, reaching one half maximum growth at approximately 0.1 μg. of thiamin per 25 ml. The responses of strains 1090 and 17084 are considerably poorer, but these reach practically normal weights with a sufficient amount of thiamin. In many experiments strain 17084 has grown somewhat slower than strain 1090, but its response to B_1 after four days is the same as that of strain 1090.

Location of the biochemical blocks in strains 9185 and 18558.—Tests for the replacement of thiamin by its components, thiazole and pyrimidine[2], were made. These tests showed that strain 18558 responds just as well to thiazole (fig. 1), while strain 9185

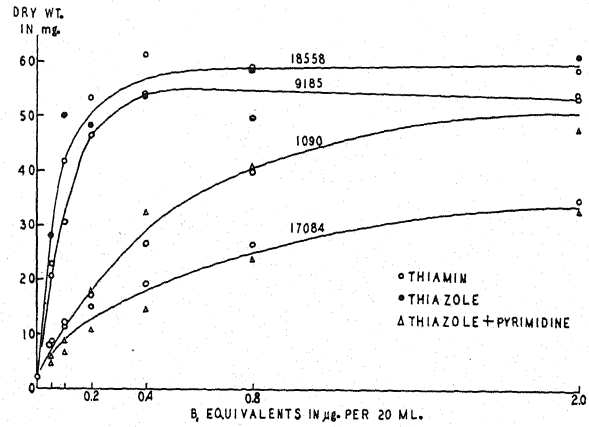


Fig. 1. Growth responses of *Neurospora mutant* strains to thiamin and thiamin intermediates.

requires the intact thiamin molecule. The results suggest that strain 18558 is unable to synthesize the thiazole component, while strain 9185 cannot couple the two intermediates. Each strain differs from normal in a single gene and therefore in only one reaction if the expected one-to-one relation of gene to reaction (Horowitz *et al.*, 1945) holds. Reactions in the biosynthesis of thiamin other than the genetically blocked one should then be expected to take place normally. One might then predict the formation of pyrimidine by strain 18558, and the production of both components by strain 9185. This was tested by growing these strains in minimal medium containing a limiting amount of thiamin (0.5 μg. per 50 ml.). The cultures were grown with constant shaking, and after five days at 25°C. were heated to boiling and filtered. The filtrates were then assayed for intact thiamin (with strain 9185), for thiazole (strain 18558), and for pyrimidine and thiazole with *Phycomyces blakesleeanus* (Bonner and Erickson, 1938) and *N. crassa* strain 17084. The results shown in table 3 clearly demonstrate the synthesis and accumulation of pyrimidine by strain 18558 and that of both thiazole and pyrimidine by strain 9185. In no case did the filtrates show any appreciable activity for the strain from which they were obtained.

Thiazole synthesis by strain 18558.—The specificity of response of strain 18558 to thiazole was tested with a number of analogues. Because of the limited available amounts of certain of these, most

[2] For simplicity, the terms thiazole and pyrimidine, unless otherwise qualified, will be used to indicate these compounds substituted as in thiamin. 4-methyl, 5-β-hydroxy ethyl thiazole was obtained from Dr. E. R. Buchman, and 2-methyl, 5-ethoxymethyl pyrimidine from Merck & Co.

TABLE 3. *The production of thiamin intermediates by strains 9185 and 18558—values in body of table are expressed as B_1 in μg. per 50 ml. culture and include thiamin present.*

Strain number	B_1 added to original culture	B_1 found	Thiazole found	Thiazole plus pyrimidine found		Pyrimidine found
		Assay with N.c. 9185	Assay with N.c. 18558	Assay with N.c. 17084	Assay with Phycomyces	Assay with Phycomyces[a]
9185	0.5	0.25	12–30	17–25	20	20
18558	0.5	0.2	0.15	0.0	0.0	7.0

[a] With 2.5 μg. of thiazole added per 25 ml.

growth experiments were carried out in test-tubes containing 10 ml. of medium, incubated in a slanting position. The mycelium was harvested after five days, and washed, dried and weighed. The results are given in table 4. Only those analogues showing significant activity in concentrations corresponding to 0.1 to 10 μg. of vitamin thiazole per 10 ml. are given. A number of others were tested but were inactive at these concentrations, as has been reported for other organisms (Bonner and Erickson, 1938; Schopfer, 1943). Analogues 2 and 3 which could presumably be converted biologically to vitamin thiazole (Bonner and Erickson, 1938; Harington and Mogridge, 1940) showed appreciable activity, while others were inactive or showed little activity that could not reasonably be attributed to traces of vitamin thiazole. Analogues 4 and 6 (5-γ and β-hydroxypropyl analogues) showed some activity, perhaps due to contamination since the corresponding vitamin analogues were inactive. The 5-ethyl analogue may have true activity for *Neurospora* in contrast to *Phycomyces,* since both the thiazole analogue (number 8) and the corresponding vitamin analogue (number 9) were active. (For a discussion of this point see Bonner and Erickson, 1938.) This compound has not been previously reported to have activity for any organism. Number 10, the 2,4-dimethyl analogue, showed significant activity for *Neurospora* in contrast to its lack of effectiveness for other organisms with the exception of certain flagellates (Lwoff and Dusi, 1937). Two samples of this analogue showed similar activity, which suggests either its conversion to vitamin thiazole by removal of the 2-methyl group, or possibly its activity *per se*. These seems little possibility that any of the analogues tested could be normal precursors of thiazole in *Neurospora.*

The synthesis of thiazole from acetopropyl- or chloroacetopropyl-alcohol and thioformamide has been demonstrated for pea roots (Bonner and Buchman, 1938). These substances were tested for thiazole activity on strain 18558. Although a combination of either acetopropyl or chloroacetopropyl alcohol with thioformamide[3] was slightly active, the activity was so low (0.02–0.03 per cent of that of vitamin thiazole) that it seems improbable that either combination is involved in the normal synthesis of thiazole by *Neurospora.* In a mutant strain differing from normal by a single gene, and therefore in only one biochemical reaction, a normal precursor might be expected either to be completely inactive or to show activity of the same order of magnitude as the final product.

Thiamin synthesis by strain 9185.—The probable presence in alfalfa of a heat- and alkali-stable substance, Factor S, apparently essential in the bio-

[3] Thioformamide was synthesized by the method of Gabriel (1916).

TABLE 4. *Specificity of the thiazole requirement of strain 18558. (All analogues tested had a methyl group in position 4.)*

Number	Thiazole substituent group in positions 2	5	Quantity tested	Activity for Strain 18558	Activity for Phycomyces[a]
			μg./10 ml.	per cent	per cent
1	—H	$-CH_2CH_2OH$	0.1	100	100
2	—H	$-CH{=}CH_2$	10	1.0	0.1
3	—H	$-CH_2-CHNH_2-COOH$	10	0.3	....
4	—H	$-(CH_2)_2CH_2OH$	10	0.1	0.06
5[b]	—H	$-(CH_2)_2CH_2OH$	1000	0.0	0.06
6	—H	$-CH_2-CHOH-CH_3$	10	0.2	0.09
7[b]	—H	$-CH_2-CHOH-CH_3$	1000	0.0	0.1
8	—H	$-CH_2CH_3$	10	0.1	0.00
9[b]	—H	$-CH_2CH_3$	10	0.1	....
10	$-CH_3$	$-CH_2CH_2OH$	10	0.8	0.00

[a] Data from Bonner and Erickson (1938).
[b] Vitamin analogues.

logical coupling of thiazole and pyrimidine by the ciliates *Tetrahymena geleii* and *T. vorax* and also by the mold *Phytophthora cinnamomi* has been demonstrated (Kidder and Dewey, 1942, 1944; Dewey, 1944). The possibility that this factor is involved in the synthesis of thiamin by *Neurospora* was therefore investigated. Alkali-treated extracts of alfalfa prepared according to the directions of Kidder and Dewey (1942), as well as an extract obtained from Dr. G. W. Kidder, which had been shown by direct test to have Factor S activity, were used. These were tested both with and without added thiazole and pyrimidine for their ability to replace thiamin for *Neurospora* strain 9185. Neither extract had any effect on the growth of this strain under the conditions tried. Either Factor S is not involved in thiamin synthesis by *Neurospora,* or the inability of strain 9185 to bring about the coupling of the components does not result from a deficiency in this substance. *Neurospora* strain 9185 resembles the ciliate *Glaucoma scintillans* (Kidder and Dewey, 1942) in its failure to respond to Factor S.

Requirements of strains 17084 and 1090.—Tests of the ability of the thiamin components, thiazole and pyrimidine to replace thiamin for strains 17084 and 1090 have shown that these strains require both thiamin components[4]. The data illustrated in figure 1 show that for these strains equimolecular mixtures of thiazole and pyrimidine had approximately the activity of intact thiamin. The response to the combination was not due to the formation of thiamin during autoclaving, since such mixtures were inactive for strain 9185, which requires the intact molecule, and since the two components autoclaved separately and then added to the medium showed the same activity for strains 17084 and 1090. The thiamin requirements of these strains therefore seem analogous to those of *Phycomyces blakesleeanus* (Schopfer, 1943).

In order to determine whether these strains produced any thiamin, thiazole or pyrimidine, or related active products, cultures were grown with shaking in 50 ml. of medium containing 0.5 μg. of thiamin. The cultures were then heated to boiling, filtered, and the filtrates tested for thiamin and for thiazole with strains 9185 and 18558. Preliminary tests showed, contrary to expectation, that the filtrates of both strains contained something with the activity of intact thiamin. This activity for strain 9185 was lost on treatment with sulfite. Further tests were then made, mainly using filtrates of strain 17084 since it was derived from *N. crassa* as were both test strains. Typical results are shown in table 5. The filtrates clearly contained a factor or factors with the activity of intact thiamin as shown by the good agreement of assays with *N. crassa* strains 9185 and 18558, and with *Phycomyces blakesleeanus*. The same filtrates did not support appreciable growth of strains 17084 or 1090 and therefore presumably did not contain thiamin as such. That the inactivity for strain 17084 was not due to an inhibition of thiamin utilization was shown by the equivalence of its response to thiamin with and without added culture filtrates.

[4] *N. crassa* strain 56501 likewise has been found to require both components of thiamin.

TABLE 5. *Production of thiamin-active substance by strain 17084. Values in body of table are expressed as* B_1 *in μg. per 50 ml. culture.*

Days incubation	Assay with N.c. 9185	Assay with N.c. 18558	Assay with Phycomyces	Assay with N.c. 17084
2	.5	.4	..	..
4	2.5	3.0	..	..
6	3.2	5.0	..	..
6	5.0	8.0	7.5	.65
8	5.0	9.0	..	..
11	5.0	8.5	5.0	.7

It has been shown that the response of pyridoxinless strain 299 to limiting amounts of pyridoxin is stimulated greatly by thiamin (Stokes *et al.,* 1943). Apparently in this strain a pyridoxin deficiency results in a secondary limitation of thiamin synthesis, or perhaps alternatively in an abnormally high thiamin requirement, perhaps analogous to that of strains 1090 and 17084. Investigation of this possibility has shown that strain 299 grown with 0.1 μg. of pyridoxin per 20 ml. requires for maximum response the addition of approximately 10 μg. of thiamin, an amount in excess of the 0.4 μg. needed for maximum responses of thiaminless strains 9185 and 18558, but similar to the higher thiamin requirements of strains 1090 and 17084. A further similarity is found in the fact that strain 299 under these conditions responds only to intact thiamin or to a combination of both components. A deficiency of pyridoxin, therefore, seems to result in abnormally high thiamin or pyrimidine and thiazole requirements, possibly due to the involvement of pyridoxin in thiamin metabolism or utilization. However, pyridoxin, pyridoxal, and pyridoxamine have no effects qualitatively or quantitatively on the thiamin requirements of strains 1090 and 17084.

Inhibitory action of pyrithiamin on Neurospora.—Woolley and White (1943) have demonstrated that the inhibition of the growth of a number of microorganisms by pyrithiamin is related to their requirements for thiamin or its components. They found, among the bacteria, yeasts, and fungi investigated, that the growth of those requiring intact thiamin is inhibited by a much lower concentration of pyrithiamin than those which respond to one or both components, while those with no thiamin requirements were not appreciably inhibited. These studies were carried out with different organisms, and it seemed worthwhile to see whether this correlation between thiamin synthesis and inhibition of growth by pyrithiamin also held for the different mutant strains of *Neurospora.*

These investigations were carried out with the results given in table 6. All four mutant strains had

TABLE 6. *Pyrithiamin inhibition of different mutant strains of Neurospora and of Phycomyces. (Tested in the presence of 0.01 μg. B_1 per ml. medium, or with equivalent amounts of thiamin intermediates.)*

Culture	Minimum thiamin requirement	Index[a] when grown with			
		Thiamin	Thiazole	Thiazole and pyrimidine	Cocarboxylase
N.c. 9185	intact thiamin	140–200	....	....	50–60
N.c. 18558	thiazole	140–250	2000	2000	.....
N.c. 17084	pyrimidine + thiazole	100	[b]	2000	.....
N.s. 1090	pyrimidine + thiazole	140	[b]	2000	.....
Phycomyces blakesleeanus	pyrimidine + thiazole	800–1200	[b]	4000	.....

[a] Expressed as ratio of pyrithiamin to 1/2 the added amount of B_1 or equivalent amounts of intermediates at the point of ½ maximum growth (19).
[b] Since these cultures can use pyrithiamin as a source of pyrimidine, these tests are of no value.

approximately the same pyrithiamin:thiamin index, varying in different experiments from 100 to 250. In *Neurospora* there therefore seems to be no definite correlation between capacity for synthesis of thiamin and its components and the degree of inhibition of pyrithiamin. The index found for *Phycomyces* of 800–1200 agrees well with the value of 800 previously found for *S. cerevisiae* (Woolley and White, 1943), which also requires both thiazole and pyrimidine. In the experiments of Woolley and White the pyrithiamin:thiamin inhibition index was determined. Sarett and Cheldelin (1944), however, found that with *Lactobacillus fermentum* and *Penicillium digitatum* the utilization of cocarboxylase was inhibited to a greater extent by pyrithiamin than was the utilization of thiamin, and concluded that free cocarboxylase was not a normal intermediate in thiamin utilization (Sarett and Cheldelin, 1944). The pyrithiamin:cocarboxylase inhibition of *N. crassa* 9185 was also found to be greater than that of thiamin, as is shown in table 6. When similar comparisons were made between thiamin and thiamin components, it was found that thiamin is much less effective in overcoming inhibition by pyrithiamin than are thiazole or thiazole and pyrimidine. In line with the reasoning of Sarett and Cheldelin regarding the pyrithiamin:cocarboxylase inhibition, this would indicate that free thiamin is not a normal intermediate in the syntheses of carboxylase or other thiamin-containing enzymes. Another, perhaps more reasonable explanation is that the utilization of endogenous thiamin synthesized in the cell from thiazole and pyrimidine is more efficient than the utilization of exogenous thiamin, and as a consequence more pyrithiamin is required to inhibit the utilization of thiamin synthesized *in vivo*. This relationship might also indicate a qualitative as well as a quantitative difference in the metabolism of endogenous and exogenous thiamin.

DISCUSSION.—The evidence obtained with *Neurospora* strains 9185 and 18558 is consistent with the view that the synthesis of thiamin in *Neurospora* takes place through the production of vitamin thiazole and pyrimidine, with their subsequent coupling. It is more difficult to interpret the requirements of the other two strains. The results showed that strains 1090 and 17084, which differ from wild type in a single gene, require thiamin or a mixture of both components to grow. By analogy with the interpretations of the requirements of other mutant strains of *Neurospora* this would suggest that in these strains the syntheses of both components are blocked by single gene mutations. This would be difficult to interpret on the basis of the existence of a common step or reaction in both processes, as has been suggested for the relation of iso-leucine and valine (Bonner *et al.*, 1943). The production by strain 17084 of a substance with the specific activity of intact thiamin, proves that compounds with structures at least very close to those of the vitamin components are produced by this mutant strain under the experimental conditions used.

One possibility is that in *Neurospora* the synthesis of thiamin does not normally proceed through formation of thiazole and pyrimidine as such, with their subsequent coupling. This does not seem likely in view of the equivalence of activity of the components and the vitamin for *Phycomyces* and other organisms, and the production of the expected components by *N. crassa* strains 9185 and 18558.

A possible alternative solution is that there is some abnormality in the metabolism of thiamin in strains 1090 and 17084, possibly resulting from partial genetic and biochemical blocks in the utilization of thiamin. This may be supported by the significantly higher thiamin requirements of these strains as compared to strains 9185 and 18558. The failure of these strains to grow in the absence of added thiamin might then be due to their inability to make enough vitamin precursors and therefore thiamin to meet their higher requirements, and not to genetic blocks in the synthesis of thiamin. Both these strains differ from strains 9185 and 18558 in that they will in time (15–20 days) grow quite appreciably on minimal medium, suggesting a slow synthesis of thiamin. The product formed by strain 17084 might be a metabolic product closely related to thiamin and much more efficiently utilizable by strains 9185 and 18558 and by *Phycomyces* than by strains 1090 and 17084. The formation of this substance would result in the higher thiamin requirements of strains 1090 and 17084,

as well as in the inability of these strains to grow normally in unsupplemented media.

SUMMARY

Four mutant strains of *Neurospora* which require thiamin for growth have been found to be differentiated from wild type by single genes.

The synthesis of thiamin in *Neurospora* strain 18558 has been shown to be blocked only in the synthesis of thiazole, and that in strain 9185 only in the coupling of the two components. The synthesis and accumulation of pyrimidine by strain 18558 and of both pyrimidine and thiazole by strain 9185 have been demonstrated.

Mutant strains 17084 and 1090 have been found to require for growth either thiamin or a mixture of thiazole and pyrimidine. Strain 17084 has been shown to produce a substance with the specific activity of intact thiamin for *Neurospora* strains 18558 and 9185 and for *Phycomyces*.

The evidence suggests that the thiazole analogues tested, and combinations of aceto-propyl and chloracetopropyl alcohols with thioformamide, are not normal precursors of thiazole in *Neurospora*. The synthesis factor of Kidder and Dewey has no demonstrable activity in *Neurospora*.

There seems to be no relation between the type of thiamin deficiency in mutant strains of *Neurospora* and the inhibition by pyrithiamin of their response to thiamin. The utilization of cocarboxylase is inhibited to a greater extent than that of thiamin, while the utilization of thiazole is much less strongly inhibited by pyrithiamin.

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INTERSPECIFIC HYBRIDIZATION IN PARTHENIUM. II. CROSSES INVOLVING P. ARGENTATUM, P. INCANUM, P. STRAMONIUM, P. TOMENTOSUM AND P. HYSTEROPHORUS [1]

Reed C. Rollins

ASIDE FROM the desire to broaden the base of genetic materials from which the rubber and total shrub-bearing qualities of guayule, *P. argentatum* Gray, might be improved, other practical as well as academic considerations have motivated a rather comprehensive program of interspecific crossing work in *Parthenium*. Such factors as winter hardiness, speed of shrub growth, total size of shrub and disease resistance may affect the ultimate yield of rubber and the adaptability of any given variety or strain of *P. argentatum*. Any one or all of these factors would be expected to be altered by crossing *P. argentatum* with other species of *Parthenium*. Whether these alterations are "beneficial" depends in large part upon the desired result. The comparative value of "beneficial" alteration must be determined by experimental procedures. In any case, the first step is to make the crosses and obtain the hybrids. Refined comparative tests of the hybrids must be a separate project, involving different techniques.

The greater vigor and more rapid growth apparently characterizing many of the interspecific F_1 hybrids thus far obtained, as compared with *P. argentatum*, are indeed promising. Of less practical importance but of high scientific interest are the facts obtained concerning the relationships of the species and their probable evolution. The reproductive processes have been shown to be highly complex in *P. argentatum* and *P. incanum* (Powers and Rollins, 1945). The present information concerning *P. stramonium*, *P. tomentosum* and *P. hysterophorus* indicates that these are sexual species. Certainly the material of these species we have worked with intensively is both sexual and largely, if not completely, self incompatible.

Crosses between *P. argentatum* and *P. incanum* have been reported previously (Rollins, 1945). The present paper is concerned with crosses involving these species and *P. stramonium* Greene, *P. tomentosum* DC., and *P. hysterophorus* L. In spite of the fact that *P. incanum* and *P. argentatum* are in section *Parthenichaeta* of the genus *Parthenium*, and *P. tomentosum* and *P. stramonium* are in section *Partheniastrum*, crosses between these four species were made very readily. *Parthenium hysterophorus* is placed by taxonomists in still another section, *Argyrochaeta*. Crosses between *P. hysterophorus* and *P. argentatum* and between *P. hysterophorus* and *P. incanum* have proved to be more difficult and hybrids have been obtained on a limited basis only. Apparently *P. hysterophorus* will not cross at all with *P. stramonium* and *P. tomentosum*.

Most of the interspecific hybrids obtained within the genus *Parthenium* have involved *P. argentatum* as one of the parents. These hybrids have been studied more fully than those between other species of the genus. For this reason, more complete data are presented for interspecific crosses involving *P. argentatum* than for other crosses reported.

MATERIAL AND METHODS.—The plants of *P. argentatum* (fig. 9) used were grown from seeds gathered from wild plants in Mexico and Texas or were of commercial strains, the seeds of which were obtained from the Forest Service of the U. S. Department of Agriculture. Seeds of *P. incanum* (fig. 10) were obtained from wild plants in Arizona, New Mexico, Texas and Mexico. Both of the above species have been adequately described and illustrated (Rollins, 1944, 1945; Stebbins and Kodani, 1944).

The plants of *P. stramonium* used were raised from seeds collected six to eight miles south of Cedros in the state of Sonora, Mexico.[2] This species (fig. 12 and 13) is shrubby or tree-like, often attaining a height of 18 to 20 feet growing under natural conditions. In the greenhouse, in limited containers, the plants range from four to ten feet high after a year and a half of growth. *P. stramonium* characteristically has a central trunk with the lowest branches originating two to six feet above the ground. There is a continuous natural shedding of the lowermost leaves so that the trunk and branches are free of leaves except at the very tips. The trunk and branches are densely clothed with a stramineous tomentum. An analysis of sections of the stem of *P. stramonium* revealed that no rubber is present in the stem tissues. The chromosome number of *P. stramonium* is $2n = 36$.

Seeds of *P. tomentosum* (fig. 14) were collected near Almoloyas, 16 to 18 kilometers above Tomellin, Oaxaca, Mexico. This species is similar in many respects to *P. stramonium*, but differs in a number of characters. Some of the easily observable differences are as follows: *P. tomentosum* does not consistently shed all of the leaves along the trunk and lower portions of the branches as does *P. stramonium*. The trunk and branches of the former are relatively free from the thick stramineous tomentum and are greenish. The indument of the leaves of *P. tomentosum* is loose and cobwebby while that of *P. stramonium* is dense and tightly packed. Also, the leaves of *P. tomentosum* tend to be obtuse to

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Part of the results reported herein were obtained while the author was on the staff of the Special Guayule Research Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. D. A., Salinas, California.

[2] The seed collections of *P. stramonium* and *P. tomentosum* were made by Mr. H. S. Gentry, to whom our appreciation is here recorded.

rounded at the apex and a wing is often present along the petiole. The leaves of *P. stramonium* are narrower, tend to have an acute apex and the petiole is not winged. In spite of the indicated differences between plants of *P. tomentosum* and *P. stramonium,* it is not clear whether two species or two varieties of the same species are represented. The need for a close comparison of specimens and plants to determine the taxonomic relationships of these entities is indicated. The chromosome number of *P. tomentosum* is 2n = 36.

Parthenium hysterophorus (fig. 11) is an annual herbaceous species apparently indigenous to the West Indies and portions of the mainland of North America adjacent to the Gulf of Mexico. It is a common weed, widely distributed in the tropical and subtropical areas of the world. Seeds of this species were obtained from two locations, Del Rio and Moron, Texas. In addition to the herbaceous habit, such prominent characteristics as the highly dissected leaves and long simple trichomes are easily observed. This species as well as *P. lyratum* (*P. lyratum*[3] has not been used in crossing experiments with other species to date, and so could not be included in the present paper) have white pollen, whereas all the other species worked with have yellow pollen. The chromosome number of *P. hysterophorus*[4] is 2n = 34. Previously, the chromosome number for this species was reported by Kokieva (1929) as n = 18. However, from our counts on *P. hysterophorus* and the evidence from the closely related species, *P. lyratum,* this record appears to be incorrect.

The techniques used in making the crosses have been described in a previous publication (Powers and Rollins, 1945). The seeds were germinated between wet blotters in a germinator and were transplanted individually into 2½-inch pots. The seeds were all treated with an aqueous solution of sodium hypochlorite at the rate of 4 ml. per 100 seeds following the procedure given by Benedict (1945). This treatment was worked out to promote optimum seed germination in *P. argentatum,* but it has not proved at all satisfactory as a treatment for the seeds of *P. tomentosum* and is only moderately effectively with *P. stramonium.* It was necessary to free the seeds from the fruit coat by dissection and to puncture the seed coat in order to obtain satisfactory seed germination from these species.

Pure pasteurized sand was used as a culture medium. The plants were raised to transplantable size by keeping the sand moist with daily applications of one-fourth strength Hoagland's nutrient solution No. 1 with micronutrients added (Hoagland and Arnon, 1939). This procedure in handling the young seedlings almost, if not entirely, eliminated the problem of damping off during the early stages of growth. Also, a lower percentage of weak seedlings died when this procedure was followed than when soil or soil and sand mixed plus water were used. A further advantage of using sand and Hoagland's solution was derived from the fact that the pots could be placed over crocks with the bottom touching water without injury to the plants. Thus roots growing through the bottom of the pots into the water were excellent for cytological purposes.

Chromosome counts were made from aceto-orcein smears following the techniques described by Meyer (1945). The counts for the hybrids as given do not

[3] The chromosome number 2n = 68 has been found to be prevalent in *P. lyratum.* One plant was found with 2n = ±102 chromosomes.

[4] The chromosome number of *P. hysterophorus* was first determined as 2n = 34 from pollen mother cells by Dr. A. D. Bergner. Root-tip counts made by the author confirmed this number as being valid for several plants of each of the two cultures grown.

TABLE 1. *A comparison of the percentage of viable seed obtained from certain individual plants of P. argentatum treated by emasculation and nonpollination, by selfing, by open pollination; and when pollinated by four different species of Parthenium*[a].

Plant number	Treatment or source of pollen						
	Emasculated and non-pollinated	Selfed	Open pollinated	*P. hysterophorus*	*P. stramonium*	*P. tomentosum*	*P. incanum*
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
2n = ca. 36 chromosomes[b]							
4255-III	..	0	25.8	0	51.8	41.9	66.5
8118-68	..	0	28.4	0.6	42.3	30.2	19.5
2n = ca. 72 chromosomes[b]							
593-II	0	8.3	27.1	3.5*	...	...	56.5
593-V	0	17.9	47.9	2.9*	30.0	51.7	...
593-VIII	0	34.3	26.0*	5.3	13.3	...	...
42268-IV	0	34.1	28.2*	15.7	14.8	...	...
42440-I	0	27.8*	44.4	0.6*	19.5	...	...

[a] Over 200 seeds were used for each test of each treatment except in six instances. These are marked with an asterisk. In each of these six tests, over 100 seeds were used. This number may seem small to workers not familiar with the fact that each seed represents a separate pollination.

[b] Small or "miniature" chromosomes were present in addition to the number given.

include miniature[5] chromosomes, since their number was not constant for all the hybrids of a given cross. The presence of more or fewer of these miniature chromosomes was not correlated with observable morphological differences between the members of a given progeny. The determinations of good *vs.* degenerate pollen was made by microscopic examination of the pollen after staining with a solution of methyl blue in phenol and lactic acid. Degenerate pollen grains failed to take the stain, whereas those containing protoplasm were stained a deep blue.

EXPERIMENTAL RESULTS.—The interspecific crosses thus far made in the genus *Parthenium* and the directions in which pollen was carried are shown in figure 15. The results of crosses where *P. argentatum* and *P. incanum* were used as the female parent were similar to those obtained from crosses between the two species themselves (Powers and Rollins, 1945; Rollins, 1945) in showing that reproduction by seed may involve apomixis, reduction followed by fertilization, or non-reduction followed by fertilization. On the other hand, the reproductive cycle in *P. stramonium* and *P. tomentosum* is of the sexual type in the material studied. No data have been obtained concerning the methods of reproduction of *P. hysterophorus*, but the behavior of this species gives every indication that it reproduces sexually.

Effectiveness of different pollens.—The pollinations indicated in figure 15 were not equally successful. That such would be the case when the species of different sections of the genus were involved was entirely expected. The data in table 1 give an indication of the relative effectiveness of the pollens of different species in producing viable seed when applied to seven different plants of *P. argentatum*. The comparative data for the sexual plants No. 4255-III and No. 8118-68 of the 36-chromosome group show that pollination by *P. incanum*, *P. tomentosum* and *P. stramonium* was nearly as effective or more effective than open pollination in bringing about the formation of viable seed. The viable seed thus obtained produced interspecific

[5] See Bergner (1945) for a discussion relating to miniature chromosomes in *P. argentatum*.

TABLE 2. *Results of cross-pollinations between P. argentatum, P. hysterophorus, P. stramonium, and P. tomentosum where P. argentatum was used as the female parent in each case. Under each attempted cross the specific matings are grouped according to the approximate chromosome number of the female parent.*

Plant numbers	Seeds	Seedlings	Plants classified	Hybrids	Maternals
	number	number	number	per cent	per cent
P. argentatum × *P. hysterophorus*					
2n = ca. 36 chromosomes					
4255-III × 43699-I	325	0	0	0	0
4255-III × 43700-I	181	0	0	0	0
8118-68 × 43700-I	178	0	0	0	0
8118-68 × 43699-III	183	2	0	0	0
2n = ca. 72 chromosomes					
42440-I × 43700-II	174	2	1	0	100
593-V × 43700-II	138	4	4	0	100
593-II × 43700-II	197	7	5	0	100
593-VIII × 43699-II	284	15	12	0	100
416-II × 43700-II	399	80	65	0	100
42268-IV × 43700-II	105	19	19	5.2	94.8
P. argentatum × *P. stramonium*					
2n = ca. 36 chromosomes					
4255-III × 43691-III	746	387	384	100.0	0
8118-68 × 43691-I	579	245	213	100.0	0
2n = ca. 72 chromosomes					
42440-I × 43691-I	409	80	72	7.0	93.0
593-VIII × 43691-I	344	46	42	7.2	92.8
593-V × 43691-IV	270	81	69	24.7	75.3
42268-IV × 43691-I	277	41	38	63.1	36.9
P. argentatum × *P. tomentosum*					
2n = ca. 36 chromosomes					
1289-2 × 44735-III	130	84	82	100.0	0
4255-III × 44735-I	372	209	197	100.0	0
8118-68 × 44735-III	383	116	111	100.0	0
2n = ca. 72 chromosomes					
593-I × 44735-II	274	102	87	5.8	94.2
593-V × 44735-II	261	143	135	13.4	86.6

hybrid plants. The pollen of *P. hysterophorus* was ineffective in bringing about viable seed production. From the taxonomic point of view, the latter species is less closely related to *P. argentatum* than the other three species so that such results might have been anticipated. However, these results serve to substantiate the conclusion that *P. hysterophorus* is more remotely related to *P. argentatum* than is either *P. stramonium* or *P. tomentosum.*

The data of table 1 upon five facultative apomictic plants of the 72-chromosome group, though incomplete, show that these plants are not as specific in their pollen requirements as are the sexual plants of the species. The pollen of *P. hysterophorus* was moderately effective in bringing about seed production, and in one case, plant No. 42268-IV, the pollen of this species was as effective as that of *P. stramonium.* As shown previously (Powers and Rollins, 1945), the facultative apomictic plants are self-compatible, whereas the sexual ones are largely self-incompatible.

Hybrids obtained.—Previous experiments with *P. argentatum* (Rollins, 1945) showed that interspecific hybrids were easily obtainable when plants of the sexual strains of the species were crossed as the female parent with *P. incanum.* As shown in table 2, similar results were obtained in the crosses *P. argentatum* × *P. stramonium* and *P. argentatum* × *P. tomentosum.* All plants of the F_1 progenies were hybrids when plants of *P. argentatum* of the 2n = 36-chromosome level were used as the female parent. At the 2n = 72-chromosome level where facultative apomixis prevails, the F_1 progenies were predominantly maternal, except in a single instance where 63.1 per cent hybrids were obtained.

The lack of compatibility between *P. argentatum* and *P. hysterophorus* is amply demonstrated by the data of table 2. It was indeed surprising that even a single hybrid was obtained. However, two hybrids from *P. incanum* × *P. hysterophorus,* species equally remote in their relationships, were also obtained. These hybrids are described below. Several pollinations involving *P. stramonium* × *P. hysterophorus* and *P. tomentosum* × *P. hysterophorus* were made, but no viable seed was obtained.

Hybrids were readily obtained when pollen from either *P. argentatum* or *P. incanum* was used upon plants of *P. stramonium* or of *P. tomentosum.* Nothing unusual concerning reproduction in either of the latter species was observed. Pollinations between *P. stramonium* and *P. tomentosum* all gave hybrids when pollen was carried in either direction and the set of viable seed was very good.

Nature of the hybrids: P. argentatum × *P. stramonium.*—Three chromosome categories of F_1 hybrids were obtained from crosses between *P. argentatum* and *P. stramonium.* Thirty-six-chromosome hybrids were obtained by using plants of the 2n = 36-chromosome sexual phase of *P. argentatum* as one parent or the other. Hybrids with about 2n = 54 chromosomes were obtained in two ways. They were consistently produced when pollen from 2n = 72-chromosome plants of *P. argentatum* was carried to *P. stramonium.* When the reverse pollination was made, an occasional F_1 hybrid with 2n = 54 chromosomes resulted, though by far the largest number of hybrids obtained in this way possessed about 2n = 90 chromosomes. In the latter type, the *P. argentatum* female parent contributed 72 chromosomes because of nonreduction, and the *P. stramonium* parent contributed 18 chromosomes. In figure 2, two leaves of *P. stramonium* are at the upper left and two leaves of *P. argentatum* are at the upper right. At the lower left are two leaves of a 2n = 36-chromosome F_1 hybrid; in the lower center are two leaves of a 2n = 54-chromosome F_1 hybrid; and at the lower right are two leaves of a 2n = 90-chromosome F_1 hybrid. The progressive increase of genetic influence of *P. argentatum* is correlated with a proportional increase of chromosomes of this species in the hybrids. This is easily seen by comparing the leaves of the 2n = 36-, 54-, and 90-chromosome F_1 hybrids with those of the parental species. Plants of a corresponding series are shown in figure 7.

The 2n = 54-chromosome hybrids were similar whether they were obtained from a cross involving a 72-chromosome plant of *P. argentatum* as a female or a male parent. This may be seen in figure 5 by comparing the two plants at the left. The plant at the extreme left was an F_1 of a cross of *P. argentatum* ♀ × *P. stramonium* ♂. The plant next to it was obtained from the reciprocal cross. The two plants at the right in figure 5 are 2n = 90-chromosome hybrids. All four plants are three months old and it may be noted that the 54-chromosome hybrids show slightly greater growth than the 90-chromosome hybrids even at the stage of growth represented.

First generation hybrids between *P. argentatum* and *P. stramonium* were exceedingly vigorous in most instances and consistently outgrew plants of *P. argentatum* of the same age. This proved to be true of all three categories of hybrids. The 2n = 36-chromosome hybrids were faster growers than the 2n = 54-chromosome hybrids, which in turn grew faster than the 2n = 90-chromosome hybrids. This tendency is not clearly evident in the three-month-old plants in figure 7, but growth differentials showed up shortly after the plants became established in field plots.

There is a correlation between pollen size and chromosome number in the hybrids between *P. argentatum* and *P. stramonium* as shown in table 3. Similar results have been found in *P. argentatum* by Bergner (1944). The data of table 3 show that there were wide differences in the percentage of apparently viable pollen grains in the hybrids of the different chromosome groups. The 36-chromosome hybrids were consistently low, usually having less than 40 per cent good pollen. Hybrids of the 54-chromosome category possessed a higher per-

centage of good pollen than did those of the 36-chromosome group, but were inferior in this respect to hybrids of the 90-chromosome group. It should be recalled that hybrids of the 36-chromosome category showed greater and more rapid growth than those of the 54- or 90-chromosome groups. Therefore, the high percentage of degenerate pollen produced by the 36-chromosome plants is not associated with weaknesses affecting the growth characteristics of the plants themselves.

P. argentatum × *P. tomentosum.*—The results of crosses between *P. argentatum* and *P. tomentosum* were similar to those obtained in crosses between *P. argentatum* and *P. stramonium* except that the hybrids were, in general, less vigorous. Hybrids were obtained readily when pollen was carried in either direction. Figure 6 shows a young plant of *P. tomentosum* at the left; next to it is a 36-chromosome hybrid; in the center is a 54-chromosome hybrid; the plant to the right of center is a 90-chromosome hybrid, and a plant of *P. argentatum* is at the extreme right. Two leaves per plant from older plants of the same series are shown in figure 1.

From a pollen analysis of the 36-, 54-, and 90-chromosome hybrids, results very comparable to those given in table 3 were obtained. Plants of the 36-chromosome group showed less than 40 per cent of apparently viable pollen, while over 40 per cent, but less than 60 per cent of the pollen of plants of

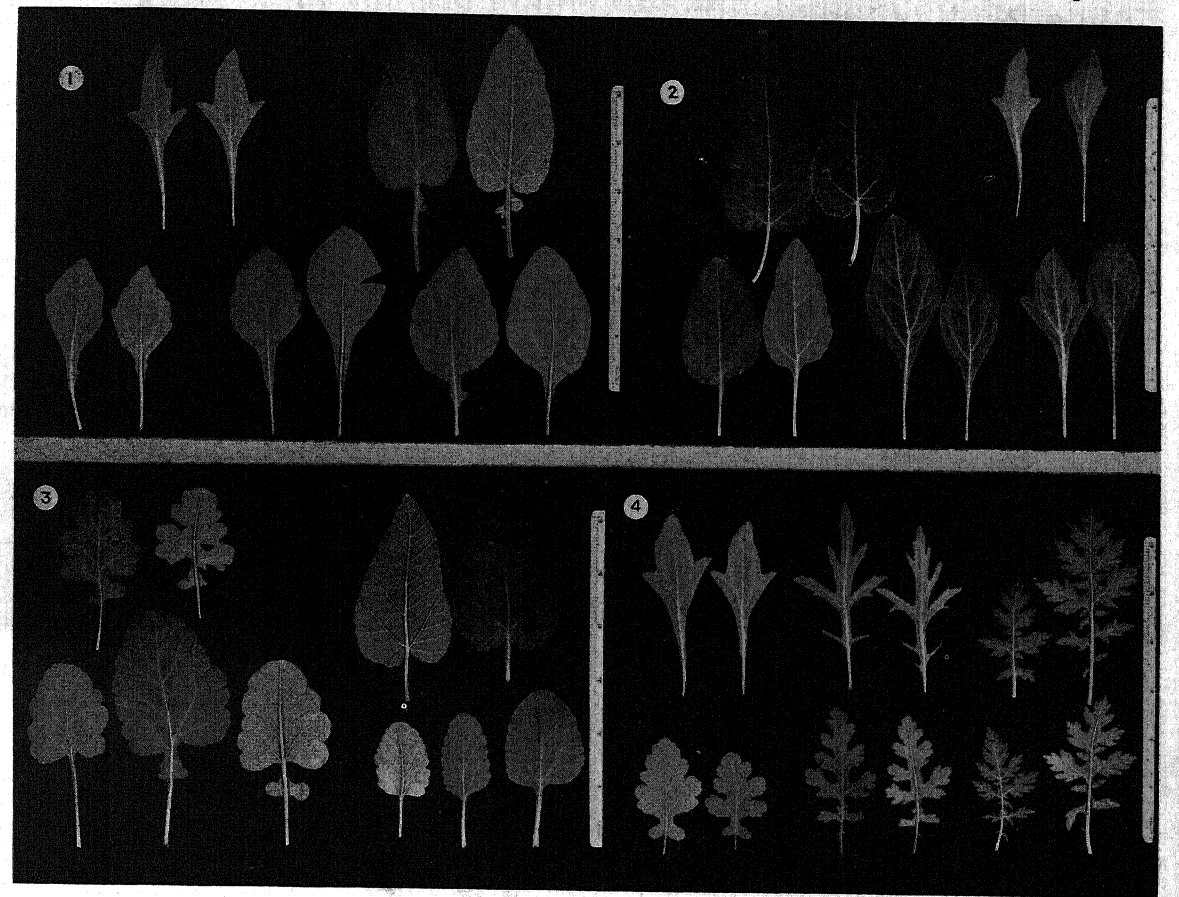


Fig. 1–4.—Leaves, showing upper and lower surfaces, from adult plants of parents and F_1 hybrids of various interspecific crosses in *Parthenium.*—Fig. 1. Parental types and hybrids of crosses between *P. argentatum and P. tomentosum.* Upper left, two leaves of *P. argentatum;* upper right, two leaves of *P. tomentosum;* lower left, two leaves of a 90-chromosome hybrid; lower center, two leaves of a 54-chromosome hybrid; lower right, two leaves of a 36-chromosome hybrid.—Fig. 2. Parental types and hybrids of crosses between *P. argentatum* and *P. stramonium.* Upper left, two leaves of *P. stramonium;* upper right, two leaves of *P. argentatum;* lower left, two leaves of a 36-chromosome hybrid; lower center, two leaves of a 54-chromosome hybrid; lower right, two leaves of a 90-chromosome hybrid. —Fig. 3. Parental types and hybrids of crosses between *P. incanum* and *P. stramonium.* Upper left, two leaves of *P. incanum;* upper right, two leaves of *P. stramonium;* lower left, three leaves of a 90-chromosome hybrid; lower right, three leaves of a 54-chromosome hybrid.—Fig. 4. Parental types and hybrids of crosses involving *P. argentatum, P. hysterophorus* and *P. incanum.* Upper left, two leaves of *P. argentatum;* upper center, two leaves of an F_1 hybrid from a cross of *P. argentatum* ♀ × *P. hysterophorus* ♂; upper right, two leaves of *P. hysterophorus;* lower left, two leaves of *P. incanum;* lower center, two leaves of an F_1 hybrid from a cross of *P. incanum* ♀ × *P. hysterophorus* ♂; lower right, two leaves of *P. hysterophorus.*

the 54-chromosome hybrids appeared to be good. The 90-chromosome hybrids possessed a relatively high percentage of good pollen, usually not less than 80 per cent and frequently better than 90 per cent. Also there was a correlation between pollen size and chromosome number in these hybrids.

P. argentatum × *P. hysterophorus*.—A single hybrid plant was obtained from a number of at-



Fig. 5–12. Plants of parental types and F_1 hybrids of interspecific crosses in *Parthenium*.—Fig. 5. Three-month-old hybrids of crosses between *P. argentatum* and *P. stramonium* growing in pots measuring four inches in diameter at the top. The two plants at the left are 54-chromosome hybrids obtained from reciprocal crosses; the two plants at the right are 90-chromosome hybrids.—Fig. 6. Five-week-old plants of parental types and hybrids obtained from crosses between *P. argentatum* and *P. tomentosum*. At the extreme left is a plant of *P. tomentosum;* at the left of center is a 36-chromosome hybrid; in the center is a 54-chromosome hybrid; at the right of center is a 90-chromosome hybrid; at the extreme right is a 72-chromosome plant of *P. argentatum*. All plants are growing in pots which measure 2½ inches in diameter at the top.—Fig. 7. Three-month-old plants of parental types and hybrids obtained from crosses between *P. argentatum* and *P. stramonium*. At the extreme left is a 72-chromosome plant of *P. argentatum;* at the left of center is a 90-chromosome hybrid; in the center is a 54-chromosome hybrid; at the right of center is a 36-chromosome hybrid; at the extreme right is a two-month-old plant of *P. stramonium*. All plants are growing in pots which measure four inches in diameter at the top.—Fig. 8. Parental types and an F_1 hybrid from a cross between *P. argentatum* and *P. hysterophorus*. At the left is a six-month-old plant of *P. hysterophorus* growing in a six-inch pot; in the center is a three-month-old F_1 hybrid growing in a six-inch pot; at the right is a three-month-old plant of *P. argentatum* growing in a four-inch pot.—Fig. 9. A two-year-old plant of *P. argentatum*. The container is nine inches across the top.—Fig. 10. A two-year-old plant of *P. incanum* growing in a container of the same size as that in figure 9.—Fig. 11. A full-grown plant of *P. hysterophorus* growing in a pot measuring eight inches in diameter at the top.—Fig. 12. A four-month-old plant of *P. stramonium* growing in a pot which measures four inches in diameter at the top.

tempts to cross *P. argentatum* and *P. hysterophorus* as shown in table 2. The maternal parent of the hybrid was a 72-chromosome plant of *P. argentatum*. The hybrid was found to possess 89 chromosomes, thus it is evident that an unreduced number came from the mother plant. The paternal plant contributed 17 chromosomes. The hybrid, shown in the center in figure 8, was considerably more like *P. argentatum* than *P. hysterophorus*. However, it bears definite characteristics which are attributable to the latter species. This can readily be seen by comparing the upper row of leaves in figure 4. The two leaves in the center are from the hybrid, those at the right are from *P. hysterophorus* and those at the left are from *P. argentatum*. The hybrid was a vigorous plant and 81 per cent of its pollen appeared to be good.

P. incanum × *P. hysterophorus*.—From several attempts to cross these two species, two hybrids were obtained. Both were derived from crosses in which 54-chromosome plants of *P. incanum* were used as the female parent. No attempts were made to make crosses in the reverse direction. The hybrids possessed about 71 chromosomes. Therefore, it may safely be assumed that chromosome reduction failed to occur preceding embryo sac formation. Thus the unreduced number of 54 chromosomes was contributed by the *P. incanum* plant in each case and the reduced number of 17 chromosomes came from the paternal plant of *P. hysterophorus*. In figure 4, two leaves from one of the hybrids are in the center of the lower row of leaves. Leaves of the maternal plant of *P. incanum* are at the left and two leaves of the paternal plant of *P. hysterophorus* are at the right. The hybrids were somewhat weaker in growth than the parental types.

P. incanum × *P. stramonium*.—Hybrids belonging to four chromosome groups were obtained from crosses between these species. Although only two to four hybrid plants of each cross were examined for chromosome number, it was evident that the chromosome number of the hybrids either represented the approximate reduced number from each parent, or the unreduced number from the maternal plant of *P. incanum* combined with the reduced number from *P. stramonium*. Hybrids from crosses *P. incanum* ♀ (54 chr.) × *P. stramonium* ♂ were all approximately 72-chromosome plants. When a 72-chromosome plant of *P. incanum* was substituted as the female parent in the same type of cross, the hybrids (at least those examined) possessed about 90 chromosomes. Three leaves of a 90-chromosome hybrid (72 chromosomes of *P. incanum* and 18 chromosomes of *P. stramonium*) are at the lower left in figure 3. A 54-chromosome hybrid is represented at the lower right in figure 3. At the upper right are two leaves of *P. stramonium* and at the upper left two leaves of *P. incanum*. The hybrids examined from the cross *P. stramonium* ♀ × *P. incanum* ♂ (54 chr.) showed a range of chromosomes from 46 to 53. Some of the hybrids from

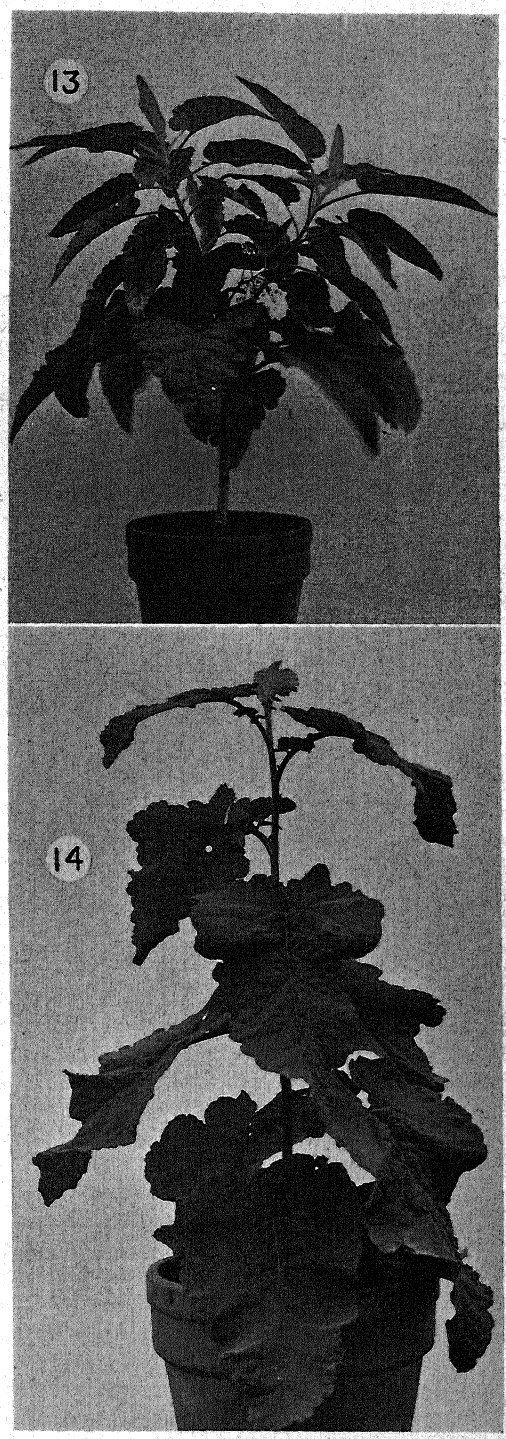


Fig. 13–14.—Fig. 13. A plant of *P. stramonium* which has flowered and subsequently branched from the leaf-axils near the base of the corymb of flower-heads. The plant is growing in a pot which measured ten inches in diameter at the top.—Fig. 14. A plant of *P. tomentosum* which has not quite reached the flowering stage. The plant is in a ten-inch pot, the same size as that of figure 13.

TABLE 3. *Six randomly selected hybrid plants from each of nine F_1 progenies of crosses between P. argentatum and P. stramonium are grouped according to chromosome number, pollen size, pollen fertility, and number of microcytes found among 100 pollen grains. The pollen classifications are based upon the tabulation of 100 grains from each plant.*

Parent plant numbers[a] and crosses made	Approximate chromosome number			Pollen diameter in microns[b]			Per cent apparently viable pollen				Number of microcytes among 100 pollen grains				
	36	54	90	13–17	17–21	21–25	20–40	40–60	60–80	80–100	0	1–5	5–10	10–15	15–20	20–25
4255-III♀ × 43691-III♂	6	..	..	6	..	..	6	..	..	..	4	2	..	..	..	..
8118-68♀ × 43691-I♂	6	..	..	6	..	..	6	..	..	..	6	..	..	..	..	..
43691-I♀ × 8118-68♂	6	..	..	6	..	..	6	..	..	..	5	1	..	..	..	..
43691-IV♀ × 4255-III♂	6	..	..	6	..	..	4	2	..	..	5	1	..	..	..	..
43691-I♀ × 593-VIII♂	..	6	..	..	6	..	..	5	1	..	..	3	3	..	..	..
43691-II♀ × 42268-IV♂	..	6	..	..	6	..	1	4	1	..	..	1	4	..	..	1
43691-IV♀ × 593-V♂	..	6	..	..	6	..	..	6	..	..	..	1	4	1	..	..
593-V♀ × 43691-IV♂	..	..	6	..	..	6	..	..	..	6	..	1	1	3	1	..
42268-IV♀ × 43691-II♂	..	..	6	..	..	6	..	..	5	1	..	..	..	..	2	4

[a] No. 4255-III and 8118-68 = 36-chr. *P. argentatum;* No. 593-V, 593-VIII and 42268-IV = 72-chr. *P. argentatum;* No. 43691-I, -II, -III, -IV = *P. stramonium.*
[b] Pollen measurements do not include the spiny exine.

crosses between these species were either dwarfed or were weak growers. However, there were many vigorous plants in every culture.

P. incanum × *P. tomentosum.*—Only two pollinations were made between these two species; 54-chromosome *P. incanum* ♀ × *P. tomentosum* ♂ and *P. tomentosum* ♀ × *P. incanum* ♂ (54 chr.). The hybrids examined from the first cross possessed about 72 chromosomes and showed considerably more influence from the maternal parent, *P. incanum,* than from the paternal parent. Although the culture was studied in an attempt to find possible hybrids which might have possessed the reduced number of chromosomes from the maternal parent, none was found. However, such plants are more difficult to recognize in crosses involving *P. incanum* than in crosses where *P. argentatum* is used as the maternal parent. For this reason, hybrids of this type might have been overlooked.

Only three hybrids of the cross *P. tomentosum* ♀ × *P. incanum* ♂ were examined for chromosome number. They were found to have between 47 and 49 chromosomes. Evidently, the reduction of chromosomes was irregular in the *P. incanum* parent.

P. stramonium × *P. tomentosum.*—Hybrids were readily obtained in reciprocal combinations of these closely related species (or possibly varieties of the same species). The within-progeny variation in each case was very marked, showing the type of segregation ordinarily found among the hybrid offspring of sexually reproducing plants. The hybrids, in general, were vigorous and rapid in growth comparing favorably in this respect to both parents. However, there was a range of variation among the plants of a given progeny in this as in other characteristics. The hybrids examined proved to possess 2n = 36 chromosomes.

DISCUSSION.—A rough measure of the compatibility of the different species of *Parthenium* dealt with has been obtained. However, the incompatibilities to crossing are not always on the species

Fig. 15. Crosses in the genus *Parthenium* which have yielded viable seeds and F_1 hybrid plants. The point of the arrowhead indicates the direction in which pollen has been carried in making successful crosses.

level so that the data bearing on incompatibility must be interpreted with care. Using *P. argentatum* as a point of reference, it is evident that *P. incanum* is the species most nearly related, for crossing occurs in nature between these species and crosses may be made freely in many combinations in the laboratory (Rollins, 1945). Little or no decrease in pollen fertility, vigor of first and second generation offspring, or seed viability was found in the progenies of crosses between these two species.

The pollen of *P. stramonium* and *P. tomentosum* was about equally effective in bringing about the production of viable seeds in *P. argentatum* and hybrids were very readily obtained in either case in reciprocal combinations. The hybrids were vigorous and showed no evidence of unusual physiological weakness. However, pollen analyses showed that a high percentage of aborted pollen was present in the 36-chromosome hybrids. The 54-chromosome hybrids showed less aborted pollen than the 36, and the 90-chromosome hybrids possessed a high percentage of nonaborted pollen. This is taken to mean that incompatibility factors were operative at the lower chromosome levels which were counteracted by the piling up of a disproportionate number of *P. argentatum* chromosomes in the higher chromosome hybrids. Data showing this are presented for hybrids between *P. argentatum* and *P. stramonium* in table 3. Similar results were obtained for hybrids of *P. argentatum* × *P. tomentosum* crosses. Thus it is clear that neither *P. stramonium* nor *P. tomentosum* is freely compatible with *P. argentatum* in spite of the fact that F_1 hybrids are readily obtainable. It is interesting that very few microcytes were found among the pollen grains of the 36-chromosome hybrids where the greatest number of aborted grains was present. Conversely, there were relatively large (see Powers and Gardner, 1945) numbers of microcytes present among the otherwise high percentage of good pollen grains of the 90-chromosome hybrids. It appears from this that the formation of microcytes is not tied up with incompatibility between the species, but rather is correlated in some way with the chromosome number present in the hybrids.

The attempted crosses between *P. argentatum* and *P. hysterophorus* were unsuccessful at the 36-chromosome level, but a single hybrid was obtained from crosses where 72-chromosome *P. argentatum* was employed as the female parent. Incompatibility appears to be sufficient to prevent hybridization between the sexual phase (36-chromosome) of *P. argentatum* and *P. hysterophorus,* but incompatibility between the species can scarcely be complete; otherwise, a hybrid, even with a high proportion of *P. argentatum* chromosomes, would not be obtainable. It may be assumed that such deficiencies or other types of incompatibilities as are present at the lower chromosome level are covered by the reduplicated chromosome set from the *P. argentatum* parent. Evidently the right combination is infrequent, for if incompatibilities were not operative at the higher chromosome level also, more hybrids should have been obtained. The yield of hybrids from crosses between *P. incanum* and *P. hysterophorus* was also very low. The two hybrids obtained possessed reduplicated chromosome sets from the *P. incanum* parent, and there is every indication that incompatibilities between the species were operative even under these circumstances. It is noteworthy that the hybrid involving *P. argentatum* and the two hybrids involving *P. incanum* represent crosses between woody shrubs and an herbaceous annual species. Such interspecific crosses are apparently rarely obtained. It should be recalled that attempted crosses between *P. stramonium* and *P. hysterophorus* and between *P. tomentosum* and *P. hysterophorus* gave entirely negative results. I believe it is safe to conclude from the studies involving *P. hysterophorus* that this species is remote in its relationship to the other four species under consideration. Apparently the few hybrids derived from crosses with this species were obtainable only because of the polyploid condition of the female parents used and because nonreduction of the chromosomes followed by fertilization was a common method of reproduction in them.

The readiness with which hybrids may be obtained from crosses between *P. stramonium* and *P. tomentosum* is one indication that these are closely related species. Casual examinations of the pollen of hybrids of these species showed that the amount of aborted pollen present was about the same as that found in the parents, that is, less than 10 per cent. This further indicates a close relationship. The systematic position of these two entities has not been the subject of special study and it may ultimately be found that they are varieties of the same species rather than distinct species.

The results of crosses between *P. argentatum* and *P. incanum* and other species of *Parthenium* further demonstrate the validity of the conclusions drawn in previous work (Powers, 1945; Powers and Rollins, 1945; Rollins, 1945) on the methods of reproduction in these species. No additional comment is required. However, the data and observations on crosses involving *P. stramonium, P. tomentosum* and *P. hysterophorus* indicate that these species reproduce by sexual means. No evidence that apomixis is present in any one of the three was apparent.

Analyses of rubber content and growth performance have not been made upon the F_1 hybrids reported in the present paper. Experiments concerning these characteristics require facilities not at present available to the author. General observations on the growth of the hybrids where *P. argentatum* was used as one parent, show that the F_1 hybrids grow faster and larger than plants of straight *P. argentatum* of the same age. Hybrids from crosses between *P. argentatum* and *P. stramonium* appear to be more promising from the point

of view of vigor, size and speed of growth than those from the other crosses reported. Naturally, the question as to rubber content is paramount to the ultimate ultilization of these hybrids, since *P. stramonium* is known to have little or no rubber present in its cells. However, from a theoretical point of view, the rubber content of the 90-chromosome hybrids should be somewhere near that of the original *P. argentatum* parents used. This is so because 72 of the 90 chromosomes came from the latter species. Although the method of reproduction prevalent in the 90-chromosome hybrids has not been determined, they should reproduce largely by apomixis. If such is the case and the rubber content has not been materially lowered from that of *P. argentatum,* some of these hybrids should be tested against cultivated strains of *P. argentatum* for their utility value.

SUMMARY

Vigorous hybrids were readily obtained from crosses between *Parthenium argentatum* and *P. stramonium, P. argentatum* and *P. tomentosum, P. incanum* and *P. stramonium, P. incanum* and *P. tomentosum,* and *P. stramonium* and *P. tomentosum.* One hybrid was derived from a cross *P. argentatum* ♀ × *P. hysterophorus* ♂ and two hybrids were obtained from crosses between *P. incanum* ♀ × *P. hysterophorus* ♂. Chromosome counts on all three of the latter hybrids showed that an unreduced number of chromosomes had been contributed by the female *P. argentatum* or *P. incanum* parent. The evidence clearly showed that *P. hysterophorus* is too remote in its relationship to the other species of *Parthenium* given above to intercross freely with them.

By using 2n = 36- and 72-chromosome plants of *P. argentatum,* a series of hybrids with approximately 2n = 36, 54 and 90 chromosomes was obtained from crosses with both *P. stramonium,* which has 2n = 36 chromosomes, and *P. tomentosum* which possesses the same number. The 36-chromosome hybrids were intermediate between the two parents. They were vigorous, but showed low pollen fertility. The 54-chromosome hybrids were more like *P. argentatum* than the other parent and the 90-chromosome hybrids were even more like the latter species. The 90-chromosome hybrids possessed a high percentage of normal-looking pollen and the 54-chromosome hybrids were intermediate between the 90- and 36-chromosome hybrids in this respect. Hybrids of all three chromosome categories were faster in growth and grew larger than plants of comparable age of *P. argentatum.*

Where plants of *P. incanum* with 2n = 54 and 72 chromosomes were used in crosses with *P. stramonium,* hybrids with about 2n = 46–53, 54, 72 and 90 chromosomes were recovered. Many of these hybrids were very vigorous, but there were a number of weak growers. Hybrids between *P. incanum* and *P. stramonium* were not subjected to any special analyses.

The chromosome numbers 2n = 36 for *P. stramonium,* 2n = 36 for *P. tomentosum,* 2n = 68 and ca. 102 for *P. lyratum* are the first reports of chromosome numbers for these species of *Parthenium.* The chromosome number of *P. hysterophorus* is 2n = 34. The number for the latter species has previously been reported to be n = 18 (2n = 36).

Hybrids with 2n = 90 chromosomes from crosses between 72-chromosome *P. argentatum* and *P. stramonium* were considered to be the most promising for immediate utilization tests for rubber production.

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NEUROSPORA. IV. A TEMPERATURE-SENSITIVE RIBOFLAVINLESS MUTANT [1]

Herschel K. Mitchell and Mary B. Houlahan

THE SECOND paper of this series (Beadle, 1945b) presents some details on the production and isolation of a number of mutants of *Neurospora*. In most cases investigated, the new strains differ from the wild type parent by a single gene and this gene is normally concerned with a step in the sequence of reactions leading to the production of an essential growth substance. It is considered that the gene controls the production of a specific enzyme that in turn catalyzes a specific reaction. As a rule, the mutations obtained in *Neurospora* appear to involve a complete inactivation or an actual physical deficiency in one gene, but recent studies on a group of temperature-sensitive mutants suggest that intermediate modifications of genes also exist.

Only one mutant has been found that requires the vitamin riboflavin (isolation number 51602), and this requirement is a function of the temperature at which the mold is allowed to grow. That is, it grows and synthesizes riboflavin at a rate approaching that of a wild type strain at temperatures below 25°C. while an external source of the vitamin is necessary above 28°C.

The present paper is concerned with the inheritance of this mutant type and its growth and its temperature characteristics as well as some consideration of gene action in riboflavin synthesis in *Neurospora*.

GENETIC ANALYSIS.—Mutant 51602 was crossed to wild type and the eight spores from each of twenty asci were isolated in order. In all twenty cases the temperature-sensitive, riboflavin-requiring mutant was recovered from four spores and wild type from the other four. Crosses to four mutants known to differ from wild type by a single gene gave similar results, and here too the mutant was recovered intact in its temperature-sensitivity and nutritional requirements from four of the spores in each of 80 asci. Therefore, it may be concluded that mutant 51602 differs from wild type by a single gene. Experiments on linkage will be reported in a general paper on this subject.

GROWTH.—Experiments on growth were carried out using the same general technique that has been extensively applied in this laboratory (Horowitz and Beadle, 1943; Beadle, 1944). Twenty ml. of medium contained in 125 ml. Erlenmeyer flasks were inoculated with a dilute conidial suspension and the mold allowed to grow at designated temperatures and periods of time. The mycelial pads were then removed, pressed on filter paper, placed in order on cardboard or filter paper, dried for three hours at 85°C., cooled and finally weighed. The medium, containing inorganic salts, sucrose, biotin and water, has been described a number of times (Horowitz and Beadle, 1943; Beadle, 1945b). The appropriate supplement required to produce growth of the mutant was added in 2 ml. or less of water.

At 31°C. mutant 51602 does not grow without addition of riboflavin to the minimal medium that supports growth for wild type *Neurospora*. With increasing quantities of added riboflavin an S-shaped growth curve is obtained. The range of concentration varies with the temperature but extends from approximately 0.1 to 2.5 micrograms of riboflavin. Growth curves for various temperatures can be determined from the data represented in figure 1.

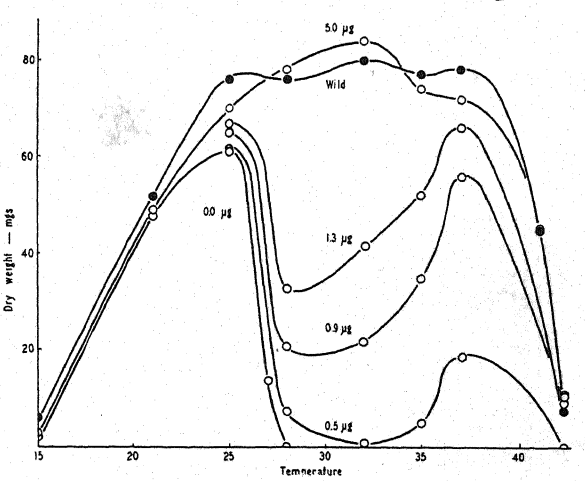


Fig. 1. Growth-temperature relations for wild type and mutant 51602. Quantities of riboflavin supplement for the mutant are indicated on the curves in terms of micrograms per 20 ml. culture. The wild type curve for Abbot 4A is indicated by the solid dots.

The mutant does not respond to riboflavin combined in the form of riboflavin-adenine dinucleotide or to the compounds 3, 4-dimethylphenyl-d, 1′ ribamine and 2-amino-4, 5-dimethylphenyl-d, 1′ ribamine. Riboflavin-5′-phosphoric acid ester has not been available in a pure form, but growth of the mutant was supported by a crude preparation made by benzyl alcohol extraction of the non-esterified riboflavin from yeast extract according to the procedure of Emmerie (1938).

TEMPERATURE SENSITIVITY.—By measuring the rate of progression of a mycelial frontier along an agar surface, Ryan *et al.* (1943) found that the growth of wild type *Neurospora* is a linear function of temperature over the range of 5° to 36°C. The rate then drops rapidly to zero at 43°C. A

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The compounds lumichrome, lumiflavin, 3,4-dimethylphenyl-d, 1′-ribamine and 2-amino-4, 5 dimethyphenyl-d, 1′-ribamine were obtained through the courtesy of Drs. Jackson W. Foster and Max Tishler of Merck and Company.
Riboflavin-adenine dinucleotide was generously furnished, from a very small supply, by Dr. F. M. Strong of the University of Wisconsin.

TABLE 1. *Riboflavin synthesis and growth of wild type and mutant 51602.*

Culture	Temperature of growth °C.	Maximum dry wt.—mgs.	Time for maximum weight—hrs.	Riboflavin added μg.	Riboflavin content μg.
Wild	25	104	135	0	6.3
Wild	34	94	130	0	8.9
51602	25	98	175	0	7.9
51602	34	0	500	0	0
51602	34	105	235	0.3	7.9
51602	34	106	205	0.6	7.2
51602	34	110	190	0.9	8.9

considerably different result is obtained from growth experiments in liquid medium during an 84-hour period. The curve connecting the black dots in figure 1 represents the average of five determinations using wild type strain Abbot 4A, the irradiated parent of mutant 51602. Figure 1 also illustrates the growth-temperature relationship for the mutant grown on a medium supplemented with the designated quantities of riboflavin. Several significant facts concerning these curves should be noted. The mutant grows in a nearly normal fashion without added riboflavin up to 25°C. but about 28° an external supply of the vitamin is required. This is the case even up to a 500-hour growth period. From the curves representing the effect of limiting quantities of added riboflavin, it is evident that a large variation exists in sensitivity to added supplement. That is, 0.9 micrograms of the vitamin supports only about 20 per cent maximum growth at 30°C. while the same quantity supports 70 per cent maximum growth at 37°C.

ADAPTIVE CAPACITY OF THE MUTANT.—Since strain 51602 is capable of growth on the minimal medium up to 28°C., it would appear that riboflavin is synthesized at the lower temperatures. This has been confirmed experimentally. In the temperature range above 28°C. it has been shown that growth is a function of the amount of added vitamin when the time is limited to that usually sufficient for nearly maximum growth of *Neurospora* under the experimental conditions used. This fact has been illustrated in figure 1 for a time interval of 84 hours. However, if given sufficient time and a small "primer" of riboflavin, the mutant will continue to grow in a spasmodic fashion and eventually produce a mycelial dry weight corresponding to the maximum reached by wild type. It is, therefore, evident that the mutant has a more or less limited capacity for riboflavin synthesis over the whole temperature range. Some representative experimental data pertinent to this conclusion are given in table 1 and figure 2. It was necessary to introduce one modification in the general technique already described to obtain the data indicated. In order to make possible the determinations of the riboflavin content of the mycelial pads, the cultures were removed following growth at specified temperatures and time intervals and immediately autoclaved. This treatment served to kill the mold and allow a free diffusion of riboflavin and its derivatives throughout the medium. Dry weights of the pads were then determined as before, but the values are somewhat lower than without autoclaving due to the loss of diffusible substances. The riboflavin content of the medium from each culture was determined microbiologically using *Lactobacillus casei* according to the standard method of Snell and Strong (1939). The values are given in terms of micrograms of ribo-

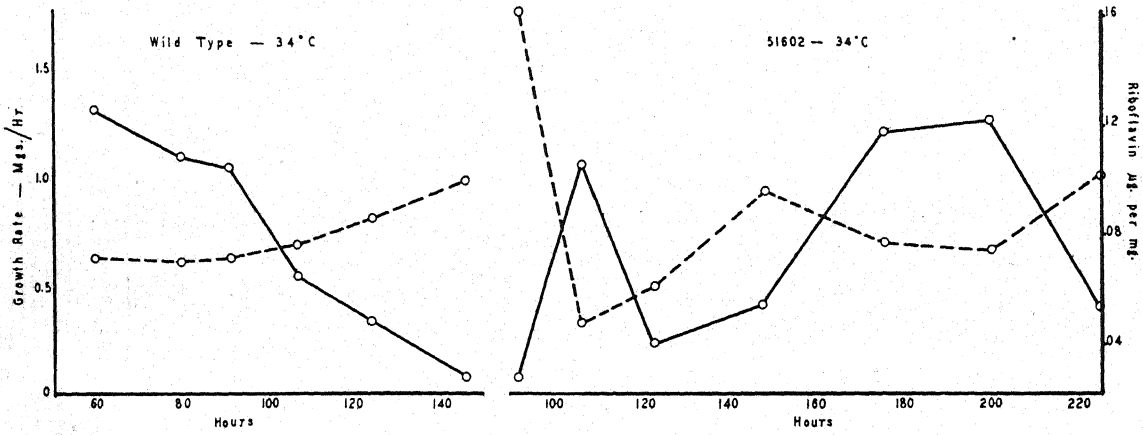


Fig. 2. Rate of growth and production of riboflavin by wild type and mutant 51602. Growth of the mutant was initiated with 0.3 micrograms of riboflavin per 20 ml. of medium. The solid lines correspond to the growth rate given on the left axis and the dotted lines to the riboflavin content and the scale on the right.

flavin, although the method measures riboflavin phosphoric esters as well as the uncombined vitamin.

The evaluation of the time required for the mold to reach a maximum weight is subject to considerable error since autolysis begins while growth is still proceeding. However, the time differences are large enough so that the determination is not critical. From the data in table 1, it may be observed that mutant 51602 requires a longer time than wild type to reach maximum growth and that it synthesizes just as much riboflavin during the process. In addition it should be noted that the mutant grown at 34°C. with small and varied supplements of the vitamin reaches about the same maximum growth and riboflavin content as does the wild type. However, the time required to do so is an inverse function of the quantity of riboflavin supplied to start the process. A similar result has been obtained at 28° and 31°C., and it, therefore, appears that the quantity of riboflavin necessary to promote maximum growth does not measure the mold's requirement for the vitamin, since the compound is synthesized to some extent during growth at any temperature.

Figure 2 illustrates the sporadic growth of mutant 51602 as compared to the relatively regular growth of wild type. The corresponding changes in the riboflavin content of the mold are also presented. Both strains were grown at 34°C. and the mutant was supplemented with 0.3 micrograms of riboflavin per 20 ml. culture. The time interval covered extends from about one-tenth maximum to maximum growth. It is shown that wild type growth proceeds with a fairly regular decrease in rate and that a constant riboflavin content is maintained during rapid growth with the synthesis continuing unchecked as the growth rate falls off. The mutant, however, grows intermittently, showing an inverse relation to the riboflavin content. That is, during periods of rapid growth, the vitamin is used up, and during periods of slow growth, it accumulates. It is possible that part of this accumulated activity is due to a riboflavin precursor that is biologically active for *L. casei* and not for *Neurospora*. Although data are presented demonstrating an intermittent growth and riboflavin production for only one set of conditions, a similar result was obtained even at 25°C. at which temperature the mutant does not require an external supply of the vitamin. At this temperature the rate variations are less and of shorter duration. At 34°, with increasingly larger riboflavin supplements, the sporadic growth gradually disappears until the rate becomes nearly the same as that of the wild type at a level of 2.5 micrograms of riboflavin per 20 ml. culture.

REVERSIBLE INHIBITIONS.—In an attempt to utilize mutant 51602 in a bioassay for riboflavin, it was found that tissue extracts were inhibitory rather than stimulatory. In view of the natural occurrence of lumichrome and lumiflavin (Brunner and Boroni, 1936; Foster, 1944a, b), the ease of their formation from riboflavin under the influence of light and their structural relationships to riboflavin, these compounds seemed likely prospects as inhibitors. Tests on the two compounds demonstrated that both are inhibitory. Table 3 summarizes some experiments

TABLE 3. *Reversible inhibition by lumichrome.*

Riboflavin added μg.	Lumichrome to give half maximum growth μg.	Mols-lumichrome / Mols-riboflavin at half maximum growth
2	1.4	1.2
5	3.6	2.2
15	10.8	2.3
40	28.8	2.5

demonstrating a reversible inhibition by lumichrome in the utilization of riboflavin by mutant 51602. The data were obtained by supplying the quantities of riboflavin listed in the first column of the table to four series of flasks, each series containing increasing quantities of lumichrome. For comparative purposes lumichrome inhibition is listed in the table in terms of the number of micrograms required to depress growth to half the maximum obtainable from the quantity of riboflavin supplied. The data demonstrate a reversible inhibition if riboflavin is supplied in excess of the amount necessary for maximum growth. Fifty per cent inhibition is brought about by a molecular ratio of lumichrome to riboflavin of 2.2 to 2.5 while a ratio of 6 to 8 is required for complete inhibition. A plot of inhibition data in terms of v/v_i against I^2 in accordance with the equation of Ebersole *et al.* (1944) gave a series of diverging straight lines with the origin at $v/v_i = 1$ and $I = 0$. Since it has been shown that the mutant synthesizes variable quantities of riboflavin during growth, the determination of v and v_i is subject to some error. However, this deviation is very small for a short growth period and a competitive inhibition is strongly indicated. Lumiflavin is only about one-twentieth as effective as lumichrome. The inhibition experiments were carried out at 31°C. for a period of 84 hours.

For inhibition studies, tissue extracts were prepared by allowing fresh tissue to autolyze for 24 hours under toluene followed by extraction with hot water. Determinations were made of the quantities of the extracts necessary to produce a fifty

TABLE 4. *Inhibition by tissue extracts.*

Extract	Riboflavin in μg./gm. of moist tissue	Mgs. of moist tissue for 50% inhibition
Rabbit liver	27	5
Swiss chard	10	25
Milk	1.6	40
Difco yeast extract[a]	50[a]	8[a]

[a] Values given are on a dry weight basis

TABLE 5. *Reversible inhibition by chard extract.*

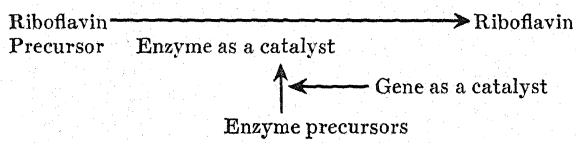
Added riboflavin μg. per culture	Mgs. moist chard for 50% inhibition
2	25
5	80
10	180

per cent inhibition of the growth stimulation normally produced by one microgram of riboflavin. Results on several extracts are listed in table 4 along with the riboflavin content of each extract. The latter values were obtained by bioassay with *L. casei*.

Reversible inhibition by chard extract is indicated by the data in table 5. The quantity of extract necessary to give fifty per cent inhibition is roughly proportional to the amount of riboflavin supplement. These experiments were also carried out at 31°C. during a time interval of 84 hours.

Experimental evidence has not borne out the supposition that the tissue extracts contain lumichrome or lumiflavin. Continuous ether extraction of liver and chard extracts did not yield an inhibitor or fluorescent compounds in the ether fraction. Judging from the experiments of Foster (1944a) these compounds should be extracted by ether under the conditions used. The nature of the inhibiting materials in tissue extracts has not yet been determined.

DISCUSSION.—In accordance with a present view on the mechanism of gene action (Beadle, 1945a), a specific step in the process of riboflavin synthesis by a wild type strain of *Neurospora* may be represented as follows:



It has been shown that mutant 51602 differs from the parent wild type strain by a single gene and that this gene is concerned with the biosynthesis of riboflavin. It is to be expected, therefore, that the mutation involves an inactivation of a gene controlling the production of an enzyme as illustrated in the diagram above. However, it has also been demonstrated that, following the accumulation of a mycelial mass under the influence of a small external source of riboflavin, the vitamin can be synthesized intermittently but in adequate quantities for eventual maximum growth. A possible explanation of this phenomenon rests on the assumption that the gene involved has not been completely inactivated, but has been altered so that it controls the production of a modified and temperature-sensitive enzyme. Another possibility that cannot be excluded requires that the gene is completely inactivated and that the temperature-sensitive riboflavin synthesis is due to the adaptive capacity of some other system in the organism. In such a case, the maximum growth that is eventually attained with a sub-optimal quantity of riboflavin may depend on a catalytic action of the vitamin combined with a partial activity of a riboflavin precursor. The finding that five "temperature" mutants of different genotypes show the striking change in synthetic ability between 25° and 28°C. (Mitchell and Houlahan, unpublished) suggests the possibility that *Neurospora* contains a normally functioning double set of genes for controlling certain reactions. One set would function up to 28°C. and the other above this temperature or over the whole temperature range. A choice of an explanation for the existence of temperature-sensitive mutants that will satisfy genetic, biochemical and evolutionary considerations cannot be made at present. Further studies on the enzymes themselves as well as on non-temperature mutants and their temperature-sensitive alleles should be revealing.

The competitive inhibition between lumichrome or lumiflavin and riboflavin is not surprising in view of the close structural relationships. However, since riboflavin and lumiflavin are isoalloxazine derivatives, while lumichrome is a derivative of alloxazine, it is unexpected that the latter compound is the more potent inhibitor. Experiments suggest that the reversible inhibition described depends upon interference in the utilization of externally supplied riboflavin. This follows from the fact that the inhibiting compounds do not affect the growth of wild type or that of the mutant when it is not dependent on an external supply of riboflavin.

The inhibition in riboflavin utilization due to tissue extracts is complicated by the fact that excessive quantities of the extracts are growth-promoting rather than inhibitory, a phenomenon that may be due to the presence of a small proportion of the total riboflavin content, as a riboflavin derivative that can be utilized without enzymatic action. Although all tissues investigated contained an excess of inhibitor over the riboflavin content, it is interesting to note that the substances are present in approximately the same proportion regardless of the total quantity. These facts suggest that the inhibiting material is derived from riboflavin.

SUMMARY

It has been demonstrated that mutant 51602 differs from wild type by a single gene that normally controls a step in the biosynthesis of riboflavin.

Riboflavin synthesis by the mutant is shown to be a function of the temperature at which the organism is allowed to grow.

A brief discussion has indicated the value of studies on mutations of this type in a further elucidation of the mechanism of gene action.

It has been shown that lumichrome, lumiflavin and tissue extracts reversibly inhibit riboflavin utilization by mutant 51602.

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GROWTH AND VASCULAR DEVELOPMENT IN THE SHOOT APEX OF SEQUOIA SEMPERVIRENS (LAMB.) ENDL. III. CYTOLOGICAL ASPECTS OF VASCULARIZATION [1]

Clarence Sterling

FEW COMPREHENSIVE studies have been made of the transition between "procambium" and "cambium" in shoot development. In fact, few reports have been concerned with the course of cambial development from its initiation at the growing point. The present paper attempts to portray this ontogeny as it occurs in the shoot of *Sequoia sempervirens*.

Dr. A. S. Crafts has suggested the use of polarized light in detecting changes in wall structure accompanying vascular differentiation. The method has been employed by Dr. Crafts (1943a) in his work on redwood and was valuable in the present investigation. Fixing and staining techniques were described in the first paper of this series (Sterling, 1945a).

DEVELOPMENT OF PROCAMBIUM AND CAMBIUM.—In *Sequoia* the young pith mother cells at the center of the shoot apex undergo vacuolation, leaving an embryonic mantle of eumeristem tissue—a fact long known to workers with gymnosperm apices. At the level of this incidence of pith differentiation, there are present usually one or two foliar buttresses and a very young foliar emergence. According to Sterling (1945a, b), there are also present leaf gaps and procambial strands, which have differentiated from the eumeristem tissue (*cf.* also Koch, 1891) but have not interrupted the general appearance of the embryonic mantle. The gap areas, although appearing early, become fully "differentiated" only after the cells of pith and cortex cease growth. (The implication of the term "differentiation" is discussed later in the paper.)

[1] Received for publication September 12, 1945.

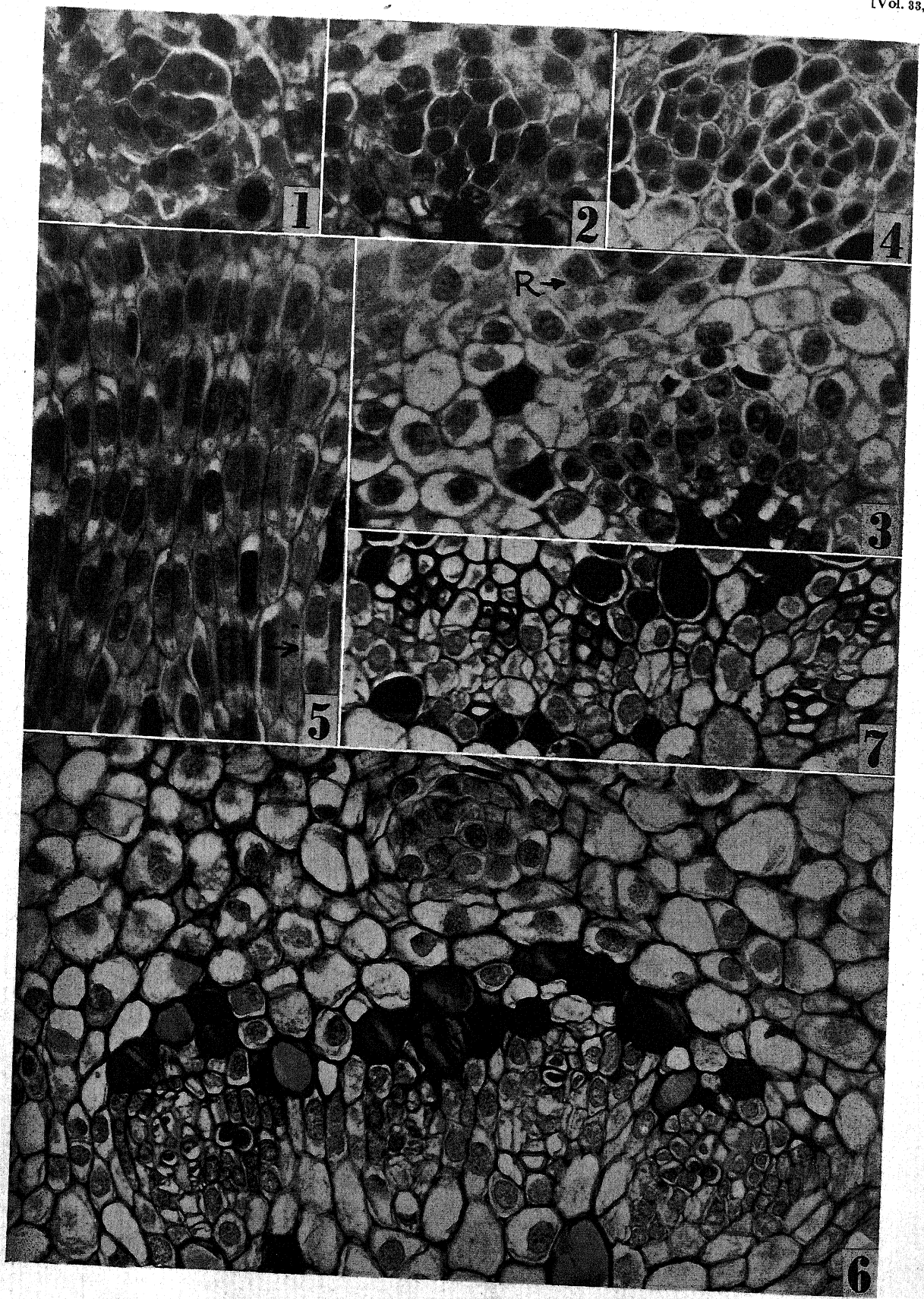
The author is grateful to Dr. Katherine Esau and Dr. A. S. Foster for their suggestions and continued interest in this work.

First stage.—In longisection, procambial tissue is first demarcated by more densely staining cytoplasm and increased rate of longitudinal division. At the same time transverse divisions become less frequent (see Sterling, 1945a, fig. 4). Soon after, elongation of the procambial cells produces their characteristically columnar form.

In transection also, the first indication of the presence of a procambial trace is the deeper staining quality of a small group of irregularly arranged cells. These at first differ little in size or shape from their neighbors in the eumeristem. Decrease in transverse diameter of the cells becomes evident as the trace develops further.

Toward the end of this initial stage in procambial development, cortical parenchyma differentiates (*i.e.*, becomes vacuolate) outside each bundle in isolated areas. The extent of this differentiation depends upon the age of the associated leaf trace. As the shoot develops, the parenchymatous areas enlarge and begin to unite. With the establishment of continuous parenchyma in the cortex, a cylinder of procambial bundles and interfascicular residual meristem (eumeristem) is roughly delimited. In this residual meristem younger traces develop acropetally from older ones along the periphery of the young pith region (Sterling, 1945b).

Second stage.—In the second stage, the component cells of the trace elongate more. Following the establishment of the locus of procambial differentiation, precise divisions occur at the periphery of the strand. In transection, the new cell walls (which are longitudinal) are oriented in apparent concentric circles about the bundle center. Other longitudinal divisions occur in the direction of radii of these circles. Thus is produced a "concentric" ar-


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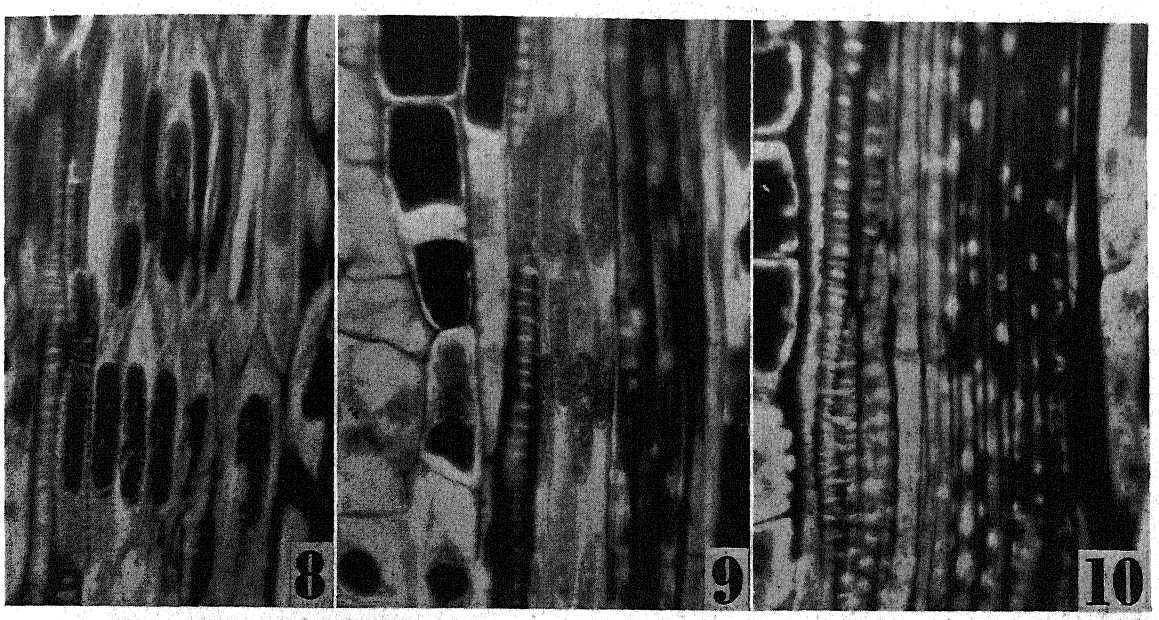


Fig. 8–10.—Fig. 8. Radial longisection of bundle at beginning of cambial transition. Nuclei can be seen in two superposed protoxylem tracheids. The nuclei in the protophloem sieve cells at the right are narrowing, preliminary to complete disintegration. ×390.—Fig. 9. Radial longisection of bundle approximately at stage of central sympodial bundle in figure 7. Cambial cells are elongated and stain lightly. The ends of two protoxylem tracheids appear to the left of the cambial cells, parts of several sieve cells to the right. Sieve fields occur in the walls of the sieve cells nearest the cambium. ×390.—Fig. 10. Radial longisection of vascular bundle with extensively differentiated secondary xylem and phloem. Last formed tracheids have bordered pits. Earliest sieve cells are obliterated. Note the changes in pattern of wall-thickenings in the radial series of tracheids. ×390.

rangement of the bundle cells (Barthelmess, 1935). By means of similarly arranged divisions in neighboring cells, the bundle is augmented in size. Cells immediately exterior to the strand are elongated parallel to the bundle periphery. These stain more deeply than cells still farther away from the bundle (fig. 1 to 3).

Radial and tangential views of the bundle at this stage show the cells arranged in stories due to a predominance of longitudinal divisions and uniform cell elongation (fig. 5). (See also Sterling, 1945a, fig. 4, and 1945b, fig. 4.) Successive longitudinal divisions seem to follow each other so rapidly that the daughter cells expand little between divisions. Although transverse divisions are diminishing in frequency, they do not cease altogether, and transverse cross walls are to be seen in almost any longisection (fig. 5). Also during this second stage, parenchyma becomes clearly defined immediately outside the leaf trace, leaving the future resin canal area as the only meristematic region dorsal to the trace (fig. 3).

Third stage.—The third stage in development is best observed in transection. This stage is characterized by the gradual orientation of new longitudinal division walls in the tangential plane. As a consequence, radial rows of cells begin to appear in the center of the bundle (fig. 4). Up to this point there has been no differentiation of distinctive phloem or xylem cells in the strand. The

Fig. 1–7.—Fig. 1. Transection of procambial bundle with concentric arrangement of component cells. ×390.—Fig. 2. Slightly later stage in development of concentric cell arrangement than in figure 1, also in transection. ×390.—Fig. 3. Transection of procambial strand somewhat older than that of figure 2. This section shows the beginning of successive tangential divisions in the center of the bundle, which will lead to radial seriation of the procambial cells. Note mitosis at the left edge of the bundle. A young resin canal (R) is developing outside this strand. ×390.—Fig. 4. Two procambial strands in transection. The more differentiated bundle at the right shows a pronounced radial seriation which immediately precedes phloem differentiation. ×390.—Fig. 5. Tangential longisection of two procambial strands at approximately the same stage of development as the bundles of figure 4. The storied arrangement of the bundle cells is clearly observable. Note transverse division wall in procambial cell at lower right (indicated by arrow). ×390.—Fig. 6. This transection (fixed in a Juel-type solution) shows somewhat later stages than the bundles in figure 4. The bundle at the extreme right shows several differentiated sieve cells (walls are very thick and gelatinous in appearance). A protoxylem element is visible on the inner border of the second bundle from the right. In the cortex can be seen a developing resin canal. ×300.—Fig. 7. Transection of a sympodial group of three bundles at the left and an isolated bundle at the right. In all, the sieve cells are radially seriated and have very thick walls. One protoxylem cell has differentiated in the central sympodial bundle. There are five tracheids in the separate strand, the last-formed of which are apparently differentiating in radial series. ×390. In all the transections, the pith is towards the bottom of the page.

TABLE 1. *Order of appearance of vascular elements in the bundles of the first leaves produced at the shoot apex (see text for further explanation).*

Distance below summit of shoot	Number of leaf (or bundle)																													
	13		12		11		10		9		8		7		6		5		4		3		2		1		—1		—2		—3		—4		—5		—6	
	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P	X	P
0 μ																																				
40 μ	0	3	0	2	0	0	0	0	0	0	0	0	0	0																						
80 μ	0	4	0	2	0	0	0	0	0	0	0	0	0	0																						
120 μ	1	6	0	2	0	0	0	0	0	0	0	0	0	0																						
160 μ	1	7	0	3	0	0	0	0	0	0	0	0	0	0																						
200 μ	1	6	0	4	0	0	0	0	0	0	0	0	0	0																						
240 μ	0	8	0	6	0	0	0	0	0	0	0	0	0	0																						
280 μ	0	8	0	5	0	5	0	0	0	0	0	0	0	0																						
320 μ	0	13	0	8	0	5	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
360 μ	1	13	0	9	0	7	0	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
400 μ	1	12	0	11	0	7	0	6	0	4	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
440 μ	0	13	0	12	0	8	0	5	0	5	0	5	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
480 μ	1	13	1	10	0	8	0	7	0	6	0	6	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
520 μ	0	12	1	9	0	11	0	7	0	7	0	7	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
540 μ	1	11	3	15	0	13	0	8	0	11	0	11	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
600 μ	3	12	2	15	0	10	0	8	0	13	0	12	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
640 μ	2	12	1	16	2	11	0	9	0	9	0	11	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
680 μ	1	14	2	13	1	10	0	10	0	13	0	14	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
720 μ	1	13	2	13	1	11	0	9	0	10	0	12	0	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
760 μ	1	15	2	13	1	12	0	14	0	13	0	19	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1	14	4	10	1	12	0	25	0	13	0	24	0	16	0	18	0	12	0	2	0	8	0	14	0	13	0	8	0	5	0	24	0	7	0	4

appearance of radial series of procambial cells occurs just previous to the differentiation of protophloem elements. The latter appear first on the outermost periphery of the bundle, hence differentiating from the cells which were originally part of the concentrically arranged group of bundle elements (fig. 8). Protophloem differentiation is continuously acropetal. (See table 1 and its explanation below). Like the procambium at this stage, the protophloem sieve cells are storied in tangential view. Tangential divisions become dominant in the inner part of the strand, so that at the end of this third stage there are usually three or four radial series of procambial cells, with five to six cells in each series.

While the bundle is augmenting its radial diameter by tangential divisions, it is also very gradually increasing its width by radial divisions. When tangential divisions first occur, radial divisions are still observable at the center of the bundle. By producing derivatives in a tangential plane, those radial divisions contribute to the storied appearance of the bundle in tangential view. The radial divisions cease in the center of the bundle when the tangential divisions begin to predominate. The former continue to persist, however, on the lateral borders of the bundle.

Although the cells of the bundle are still elongating during the third stage, they apparently do this uniformly, under the influence of shoot elongation. This suggestion is supported by the presence of the tangentially storied arrangement and by the slightly gabled end walls of these cells. The storied appearance prevails during the centripetal differentiation of one or two more layers of sieve cells, after which the procambial cells begin to elongate actively.

Fourth or transition stage.—In the fourth stage, the ends of the procambial cells begin to develop between their neighbors, the previously short-gabled ends becoming more attenuated in the process. The length of the cells increases quite markedly, as does the degree of vacuolation. At this stage there can be seen the first indications of ray initials, formed by one or more transverse divisions along the length of a procambial initial. The resulting superposed series of ray initials is at first two to several cells high. These initials separate from each other subsequently in the vertical plane. This is a physical separation caused by the vertical shortening of the individual ray initials, a phenomenon due to tangential and radial expansion of those cells. The space between the isolated initials is invaded by the neighboring fusiform cells. (Separation of the ray initials has also been indicated by Strasburger, 1891; Velten, 1875; Thompson, 1910; Bannan, 1934; and Barghoorn, 1940). The ray initials are derived from the longer procambial initials, as can be seen by their position, arrangement, and shape. They differ from the parent procambial cells in assuming gradually a more nearly isodia-

metric form, a higher nucleo-cytoplasmic ratio, a deeper staining quality, and a greater radial diameter and cross-sectional area. The cells cut off by the ray initials have relatively thinner walls than their neighbors.

Most of the radially seriated procambial cells are now differentiating into phloem elements (fig. 6, 7). There are usually only about three layers of undifferentiated cells, which are on the inner, future xylem side of the bundle. Of these, the layer next to the pith can be recognized as being part of the former group of concentrically arranged cells. During this stage, the first tracheids (protoxylem) begin to differentiate singly on the medullary edge of the bundle from these previously concentrically arranged cells (fig. 6, 7, 8, 9). Note that the first tracheids appear at isolated points. (See table 1).

Table 1 is a graphic attempt to simplify the presentation of the overall picture of vascular development in the shoot apex diagrammed in Sterling, 1945b, figure 1. The vertical columns represent the vascular bundles—these latter being assigned a numerical value corresponding to the relative ages of their respective primordia. Negative numbers of traces indicate that the primordia of the traces have not been formed at the apex. The horizontal rows indicate cross sections at succeeding intervals of 80 micra through the apex. In the bundle columns themselves, the figure "0" indicates that procambium is present at that level and that it has not yet differentiated to recognizable xylem or phloem tissue. The other numbers in the columns are the numbers of cells of differentiated phloem or xylem in the trace. At the head of the columns, the letter "X" denotes xylem elements which have a definitive, birefringent secondary wall thickening, and no cytoplast; the letter "P" indicates sieve cells which have thickened walls, staining deeply with fast green. The horizontal dotted lines across a column indicate approximate regions of union with a sympodial bundle, at which point the younger trace is considered graphically to exist no longer.

Slightly before the appearance of the protoxylem it is possible to discern a gradual increase in thickness of the radial walls of the procambial elements as contrasted with the tangential walls. In transection the elements are rectangular, with the tangential diameter slightly exceeding the radial. The rectangular shape has been produced by the restriction of most of the longitudinal divisions to the tangential plane. Thus the earliest polygonal cross-sectional form of the procambial cells existed as the concomitant of longitudinal divisions oriented in all planes. The concentric arrangement of succeeding longitudinal walls gave the bundle cells a trapezoidal shape. Then, with the formation of adjacent radial rows of tangentially dividing cells, the rectangular form was developed and maintained. The procambial cells have now become more vacuolate, this vacuolation having progressed gradually from the earlier, densely cytoplasmic stage (fig. 4 in Sterling, 1945a, and fig. 5 and 9 of the present paper form a continuous developmental series).

The observations on the developmental stages of the vascular meristem recorded above are here used to draw a somewhat arbitrary boundary between procambium and cambium. The cells of the vascular meristem are considered to have become cambial when they start intrusive growth; when their radial walls begin to thicken more than the tangential; when the ray initials are distinct from the neighboring fusiform initials; and when vascular elements (phloem elements) begin to be differentiated from cells formed by successive tangential divisions. The characteristics just enumerated are usually considered to be those of the cambium (Esau, 1943a). However, in *Sequoia,* the vascular meristem assumes cambial characteristics during rapid shoot elongation.

Fifth stage.—During the fifth stage, the interfascicular residual meristem gradually differentiates into parenchyma. These areas of residual meristem between the bundles, the so-called "pith rays," are the last portions of the residual meristem to differentiate to definitive tissue, a phenomenon noted also by Frank (1864) and Helm (1931). The cells of the leaf gaps, pith, and cortex have all differentiated at this stage to large, thick-walled, highly-vacuolated parenchyma. At the same point of development, the interfascicular areas have differentiated only partly to "definitive" parenchymatous tissue, the cells having thinner walls, smaller transverse diameter, larger nucleo-cytoplasmic ratio, and a lesser degree of vacuolation than the cells of pith and cortex. (Contrast the cells in the interfascicular areas of the four bundles in figure 6 with those of the leaf gap to the right of the group of bundles).

The cells of the interfascicular tissue which border on the vascular bundles seem to renew their meristematic activity. Some radial and tangential divisions are initiated within these cells. The renewal of cell division in the bordering interfascicular cells has been taking place continuously, serving to increase the bundle width. However, this activity is rather sluggish and does not become obvious until the interfascicular residual meristem has begun to differentiate to parenchyma. The meristematic activity is especially tardy in rapidly elongating shoots, and for several millimeters below the summit of the apical cone a bundle will have an approximately constant tangential diameter.

Within the bundle, at the start of the fifth stage, the first protoxylem cells have all matured. Succeeding tracheids are formed centrifugally and are arranged in radial rows, differentiating from the cambial derivatives laid down in radial series (fig. 7, 10). Thus, except for the production of a closed cylinder of cambial initials, the future mode of cambial activity in this region of the shoot has been fully established.

Sixth stage.—The final stage in the development of a complete cambial cylinder is the bridging of the leaf gaps and pith rays by the development of interfascicular cambium. The increase in trace width, as indicated above, has been going on continuously from the stage of the concentric cell arrangement. However, that increase is insufficient to connect the bundles across the rather wide parenchymatous areas which remain.

In parenchyma definitely recognizable as pith rays, meristematic activity, involving frequent tangential divisions, begins to take place in the cells laterally adjacent to the bundles. Thus "interfascicular wings" are formed on the bundle borders. These new wing-like extensions of adjacent bundles meet and form a continuous row of cambial initials across the medullary ray. At first, there are no xylem derivatives of the interfascicular cambium. However, sieve cells are formed which are quite similar (in transection) to the phloem produced by the fascicular cambium.

At the base of the leaf gap region, *i.e.*, in the region where a trace begins to diverge outward from the vascular cylinder, the interfascicular cambium establishes a connection between this trace and the bundles still in the cylinder. Hence, the interfascicular cambium between the departing trace and its two adjacent neighbors exists for a limited distance as an arc connecting the three across the cortical parenchyma. Still higher, as the central trace diverges farther from the ring, the cambium gradually becomes discontinuous, existing as lateral wings of the bundles bordering the gap. The wing-like extensions, in the diverging trace, are directed toward the two remaining bundles, and the extensions of these latter two are directed toward the diverging bundle.

It would appear that there is some influence existing between adjacent bundles less than a certain maximum distance apart. Possibly this influence is expressed in the differentiation of the intervening parenchyma to cambium. The differentiation is effected by longitudinal divisions in the parenchyma, the new walls being oriented in the direction of the neighboring bundles. Thus, as the abovementioned trace diverges still farther from the ring, the lateral wings of interfascicular cambium of the two bundles remaining in the ring soon begin to develop toward each other, finally establishing union across a pith ray higher up.

The bridging of the interfascicular areas was the farthest development of the cambial cylinder observed. Further cambial activity in the leaf gap areas was not studied.

DIFFERENTIATION OF VASCULAR ELEMENTS.—*Sieve cells.*—As indicated above, protophloem development is continuously acropetal (table 1). Cytologically, there are only slight differences between the sieve cells deposited at successive developmental "stages." However, since the first phloem cells will establish the poles from which succeeding elements differentiate, they can be called photophloem (Russow, 1872; Bugnon, 1924; Esau, 1943a). Although some cytological demarcation may be indicated between the protophloem and the following phloem cells, no attempt will be made to distinguish "primary" phloem from "secondary" phloem. According to current terminology, the phloem derivatives of the procambium would be designated as "primary phloem" and the phloem derivatives of the cambium as "secondary phloem." However, in the writer's opinion, the differences between the "primary" and "secondary" stages of phloem development during the first year of growth in *Sequoia* seem too slight to be indicated histologically in this study. Sieve cells following the protophloem will be designated as secondary in origin.

Naturally, the protophloem cells resemble in size and shape the procambial cells from which they were derived. They become distinguishable by an increase in wall thickness and a much higher affinity of cell wall and cytoplasm for certain stains, notably fast green.

Further development in the protophloem involves elongation of the cell, increasing vacuolation of the cytoplasm, gradual decrease in transverse diameter of the elongated nucleus which ultimately disintegrates, and a further thickening of the cell wall before nuclear decomposition (fig. 5, 6, 8). Coagulated matter in the cell lumen seems to resemble the slime plugs seen in sieve tubes of angiosperms.

The first few protophloem cells of the bundle (the outermost ones) appear to be lacking in sieve fields, a phenomenon noted also by Russow (1872) (fig. 7, 9). These sieve fields are formed only in the sieve cells which differentiate later. Thus it seems that there might be a correlation between the gradual thickening of the radial walls of the procambial cells and the incidence of sieve fields in the phloem cells differentiating from the procambial derivatives. It can be seen that when sieve fields are noticeable in the phloem cell walls, primary pit areas have become distinguishable on the procambial cell wall.[2] In fact, some radial sections may show an exact coincidence of bordered pits in the tracheids, primary pit areas in the cambium, and sieve fields in the phloem cells.

Soon after the nucleus disintegrates, the cell wall becomes thinner, the lumen is obliterated by gradual crushing of the cell, and finally the cell disappears as an entity. In some cases, a deeply staining amorphous zone on the periphery of the phloem will represent the obliterated remnants of the early sieve cells (fig. 10).

Meanwhile, "secondary" phloem tissue is differentiating from the procambial and cambial derivatives. It undergoes a much more rapid tempo of development than the protophloem, so that the wall-thickening reaches almost its fullest development

[2] Although pit areas are probably present from the first, they become recognizable only later under the fixing and staining technique of the present study.

at once even when juxtaposed to undifferentiated cambial cells. In this later-formed phloem, sieve fields are already evident. Also, in contrast to the protophloem, the thick-walled stage persists longer. Obliteration appears to take place before any marked decrease of wall thickness has occurred. This wall thickness (which is birefringent in polarized light) may become so massive that the cell lumina of the sieve elements appear as small cavities in a matrix of wall tissue (fig. 6). This condition is observable with the modification of Juel's fixative used in the present study. The wall-thickening may be comparable to the nacré thickening noted in angiosperm sieve tubes (Esau, 1939).

Tracheids.—The treatment of the xylem will parallel that of the phloem, the protoxylem being considered as a separate histological category principally because of its position as a locus of xylem differentiation. Likewise, later-formed xylem will be considered as secondary tissue. As indicated in table 1, the differentiation of the protoxylem takes place intermittently along the extent of the procambial strand on its inner face. From isolated points of origin, xylem develops acropetally and basipetally. Note that this discontinuous bidirectional type of development of the xylem is in contrast to the continuous acropetal development of the phloem (*cf.* Esau, 1943a; Crafts, 1943a, b; and Allen, 1945).

The following seems to be the sequence of development in the protoxylem: First, the cell wall of the future tracheid, at the inner periphery of the young trace, is thickened slightly by the formation of a secondary layer of wall substance uniformly over the primary wall surface. The secondary layer appears to have a high affinity for the tannic acid-ferric chloride and fast green stain. This wall thickening is very slightly birefractive in polarized light. There is only the slightest indication, in longitudinal section, of the future pattern of the secondary wall. The nucleus appears quite large and stains deeply. In transverse section, the outline of the cell becomes angular in contrast to the adjacent cells which have rounded corners.

A much thicker layer is deposited rapidly on the wall in a definite pattern, such as is recognizable in mature tracheids. This layer also stains deeply with the tannic acid-ferric chloride and fast green stain; it has little affinity for the safranin stain and is brighter in polarized light than the initial secondary layer. Longitudinal views show the thicker layer to consist of very wide bands which form a rough reticulum or a spiral with a very small helix angle. The nucleus is still clearly visible. This development takes place before the time of the pronounced shoot elongation (fig. 8).

As the rate of shoot elongation increases, the protoxylem cell becomes significantly extended. Coinciding with the gradual disintegration of the nucleus, the helix or reticulate structure is stretched, and the wall-thickenings stain more deeply with safranin. Concomitant with the new affinity for safranin dyes is a high brilliancy or anisotropism in polarized light. These last two developments indicate a rapid process of wall growth by intussusception of cellulosic and lignin materials before the expiration of the living protoplast.

Further shoot elongation stretches the protoxylem cell still more. However, in no case observed were the tracheids so far extended that the walls of the cells collapsed inwardly. Although the spiral bands might be ruptured, the transverse diameter of the cell remained very close to the diameter of the original parent procambial cell. Protoxylem tracheids are about five to nine micra in transverse diameter, whereas the procambial cells vary between eight and twelve micra. The diameter of cambial cells and later-formed tracheids averages about twelve micra in the young shoot (fig. 10).

The secondary xylem tracheids show a similar course of development, with the exception that the spiral is not drawn out as much, nor are the spiral bands as thin as those of the stretched protoxylem tracheids. Both phenomena are probably due to the decrease in the rate, and final cessation of stem elongation. It was difficult to estimate any exact correlation between the rate of stem elongation and the type of wall pattern on tracheids formed at different periods during this elongation. The later tracheids with bordered pits were shorter than the earlier spiral and reticulate elements but equal in length to the adjacent cambial cells. Fairly early in xylem formation, there are formed a few cells which show transitions between the spiral-reticulate forms and the bordered-pitted elements characteristic of the secondary xylem of *Sequoia*. Bordered pits are interspersed rather irregularly between the reticulate and spiral bands in these transition cells (fig. 10).

Vascularization in the dormant shoot.—The onset of dormancy is accompanied by the accelerated tempo of cambial activity and vascular differentiation up the shoot, so that the dormant shoot shows a pronounced development of xylem and phloem cells to about 1000 micra from the apical cone. At this level there may be four or five tracheids (including some with bordered pits) in radial rows. The cambial cylinder is almost completely closed. In actively elongating shoots, on the other hand, there are still narrow, isolated strands well below 3000 micra from the shoot summit, and for each bundle there are only two or three tracheids observable, none of which are in radial rows.

A significant feature of the dormant apex is that this increased development of cambium and vascular tissue reaches only up to the zone of transverse expansion of the pith. Above this region, the aspect of the shoot apex is almost the exact duplicate of that of the active shoot. This condition, of course, is at once reminiscent of the abietinous shoots and *Torreya*, in which a zone of thick-walled cells in the pith separates the embryonic portion of the dor-

mant bud from the more mature tissue regions. Further comparison waits upon a similar study of vascular development in the abietinous species before any definite generalizations can be made.

Discussion.—The foregoing observations on vascular development in the shoot apex of *Sequoia sempervirens* concur very closely with those of Esau on *Linum* (1942) and *Zea* (1943b). There is no procambial "ring" in this apex as postulated in studies of angiospermous plants by Kostytschew (1922, 1924) and Carstens (1931), nor is there a ring of "prevascular" meristem unassociated with the procambial bundles and interposed between these and the so-called "primordial meristem" as described by Kaplan (1936), Sárkány (1935, 1936), Helm (1931), and Bouygues (1916). However, Sárkány has noted that the meristem ring seems to occur at the level of the youngest primordia and that the ring in the *Ricinus* embryo has wide and narrow areas—according to position with respect to leaf primordia above. Sanio (1863, 1865), Russow (1872), Vöchting (1874), Halmai (1935), Louis (1935), and Popham (1941) have also related the development of a vascular ring to leaf formation. All these authors have differed in their concepts of the ring and the descriptive aspects of its development (see Helm, 1931; Esau, 1943a) but have agreed upon the fact of the presence of a more or less meristematic ring-shaped area, with which the development of procambial tissue is associated.

When the ring is delimited in *Sequoia* by cortical vacuolation, it consists of procambial bundles alternating with areas of residual meristem, the latter being a totipotent eumeristem which has not yet differentiated into definitive tissue. The acropetal development of the procambial strands into the eumeristem at the apical cone delimits a skeleton of converging isolated bundles as the first vascular development in the apex. A system of isolated procambial strands has also been noted by Frank (1864), Hanstein (1858), and Esau (1942) and has been described by Russow (1872) and Koch (1891).

Esau (1943a) has reviewed various works on the direction of procambial differentiation. She notes that although the majority of writers speak of a bidirectional differentiation from an isolated origin at approximately the leaf insertion region, a few of the more recent workers describe the procambial strands as differentiating acropetally into the leaves from older traces below. Acropetal leaf trace development occurs in *Sequoia* (also see Crafts, 1943a) and has been recently described for other plants (Crafts, 1943b; Engard, 1944; Reeve, 1942; Sass, 1944; and Allen, 1945).

Although indicating continuous acropetal procambial development in the *Cunninghamia* internode (1942), Cross depicts bidirectional development and isolated origin of this tissue in *Taxodium* (1940) and *Cryptomeria* (1941). This difference in mode of procambial differentiation in otherwise closely related genera should certainly be explored more fully.

The early concentric arrangement of cells in the procambial strand has been described by Russow (1872), Vöchting (1874), Helm (1931), Pottier (1934), Barthelmess (1935), and Esau (1942, 1943b). This arrangement is produced by concentrically oriented divisions occurring within the bundle and in adjoining cells. These divisions thus serve to add cells from the "ground parenchyma" to the procambial strand. Although such divisions occur continuously during early bundle development, they gradually cease on the tangential borders as the vascular elements differentiate. These divisions gradually become localized along the radial boundaries of the bundle, where they function to increase the lateral dimensions of the latter. This situation can also be seen in the procambial development in celery (Esau, 1936).

It must be remembered, however, that not all of the cells which are formed by the concentric divisions become bundle cells. Many become parenchymatous or present transitions between procambial cells and the surrounding parenchyma. These transitional cells may be considered to be part of the "perimedullary" and "pericyclic" zones of Flot (1893) and possibly are the source for Chauveaud's (1904) "liber précurseur" when external to the phloem tissue. However, Allen (1945) suggests that the precursory phloem is true phloem, both in position and histology. It is not unusual to find densely-staining procambium-like cells external to the protophloem in *Sequoia*.

The fact that the initiation of a radial seriation (*i.e.*, repeated tangential division) in the procambium precedes vascular differentiation has been noted by Esau (1936, 1943b) and in the present study. However, both Sharman (1942), using *Zea*, and Esau (1938, 1942), employing tobacco and flax, describe initial differentiation of both phloem and xylem before the start of radial seriation in the bundle. Many authors indicate xylem differentiation only in the cells furnished by repeated tangential divisions, mentioning the first phloem as differentiating previous to this radial seriation (Thoday, 1922; Priestley and Scott, 1936; Priestley, 1928; Popham, 1941). Engard (1944) also mentions radial seriation of the protoxylem but does not indicate the arrangement of the first phloem elements.

The early stratification of procambial cells, or "storied" arrangement, as seen in tangential view has not been extensively reported in works on procambial development. It has been described by Chen (1938), Esau (1943b), Sharman (1942), and is visible in Reeve's (1942) plate 5, figure 7, but is not mentioned in the text. In studying cambial divisions in elongating portions of young shoots (which would therefore be procambial in the sense of Esau's (1943a) definition), Kleinmann (1923)

reported radial longitudinal divisions in *Raphanus* and concluded that these divisions occurred in the cambia of all plants, including conifers. In connection with the early radial divisions in the *Sequoia* procambium, his conclusion may be accepted as valid in this particular circumstance.

No attempt will be made here to define any precise distinction between procambium and cambium. Esau (1943a) has given the salient historical viewpoints and has essayed a tentative differentiation between these two stages in cambial ontogeny based on their relationship to growth in length of the organ and cytological differences. It has been seen here how the original eumeristem cell differentiates so gradually to the mature cambial initial that there can be considerable difficulty in drawing a dividing line between procambium and cambium which would not appear artificial. Moreover, the present study suggests that the characteristics of a cambial tissue cannot be ascribed to a non-elongating shoot only (Esau, 1943a) and that the phenomena usually called cambial can also occur in a region elongating rather vigorously.

Although Kostytschew (1922, 1924) emphasized that true interfascicular cambium produces only parenchymatous tissue, this idea is contradicted by the observations of Sárkány (1936, 1937), Gráf (1938), Foster (1942, p. 111), and by those of the present writer.

The results of the present study corroborate Crafts' (1943a, b) findings on the direction of differentiation of the first vascular elements. They fit into the growing pattern of recent findings which support continuous, acropetal development of the protophloem and discontinuous, bidirectional differentiation of the protoxylem.

Despite the fact that phloem tissue of secondary origin has been extensively studied (see Esau, 1939), the cytology of the protophloem is less well known. Many recent works have supported the view of its acropetal differentiation in the vascular bundle and of its appearance previous to that of the first xylem elements (Esau, 1943a). The fact that the first indications of the protophloem sieve cells are in wall-thickening and an affinity for light or fast green stains has been recognized by Griffiths and Malins (1930), Chang (1935), Priestley and Scott (1936), Esau (1939), Sharman (1942), and Engard (1944). Crafts (1943a), Chen (1938), and Allen (1945) mention the thickened walls but apparently did not use the green stain since that is not pointed out. Most workers also agree upon the disorganization of the cell contents that soon follows the stage of pronounced wall-thickening and upon the ultimate crushing and obliteration of the sieve cell itself. Little confirmation was found in the literature for the absence of sieve fields in the first protophloem cells.

It is interesting to note that the protoxylem elements are not characterized by any fixed pattern of secondary wall deposition. The latter may vary from spiral to reticulate irrespective of the rate of shoot elongation. This is also the opinion of Russow (1872) and Bugnon (1924) (*cf.* also Esau, 1943b).

A concluding qualification must be stressed. Throughout all these phases of vascular development there is a certain degree of plasticity and individual variability—a quality inherent in all biological phenomena. This quality is expressed in the totipotentiality of the living cell, a fact which necessitates a renewed examination of the concept of differentiation.

The physiological implication of this concept is that of irreversibility of development, *i.e.*, continuous specialization of the cell in one direction. However, botanists are aware of the potentialities inherent in such a tissue as "parenchyma." The fact that parenchymatous cells can be reactivated to meristematic activity during normal development or regeneration in almost any region of the plant is well known. Whether any living cell can be said to be completely differentiated (in the sense of being unable to resume activity) is debatable. Apparently, as long as the cell has a living nucleus, it is capable to a greater or lesser extent of nuclear activity, including mitosis. The characteristics of large size, extended vacuoles, thickened walls, or the presence of ergastic material cannot be used to indicate a cell which is incapable of further changes in constitution.

SUMMARY

The process of procambial and cambial differentiation in the shoot apex of *Sequoia sempervirens* involves continuous cell elongation and vacuolation. Deeply staining procambial strands are present at the level of the pith mother cells. A concentric arrangement of the cells of the bundle (in transection) is produced by divisions parallel to its circumference. The cells are storied in radial and tangential sections because the predominantly longitudinal divisions are accompanied by apparently uniform elongation. Radial seriation is initiated by tangential divisions in the center of the strand, accompanied quickly by protophloem differentiation on the cortical edge of the strand.

Following protophloem differentiation, certain procambial cells seem to elongate more actively and begin to thrust their ends between neighboring cells, destroying the tangential storied arrangement. Ray initials become distinguishable from fusiform initials. Radial divisions are restricted to the sides of the bundle, and tangential divisions predominate in the center. Although transverse divisions decrease in frequency, they do not cease entirely. Radial walls increase in thickness more than the tangential walls, and the nucleo-cytoplasmic ratio decreases. At the same time, protoxylem develops on the inner edge of the strand. Although the cells are now called cambial, this dividing line is used only for descriptive convenience. Cambial ontogeny is

a gradual process, with no abrupt changes in development.

Tangential divisions occur on the sides of the bundle, forming an interfascicular cambium from the bordering parenchyma cells, thus closing the cambial cylinder. The first product of the interfascicular cambium is phloem.

Protophloem differentiates acropetally along the outer edge of the procambial strand from older phloem below. The walls of protophloem sieve cells stain with fast green and thicken early. There are no recognizable sieve fields of the type which occurs in the later-formed sieve cells. Obliteration of the protophloem sieve cell follows the disintegration of the nucleus and a thinning of the cell wall.

Protoxylem develops on the inner edge of the bundle, differentiating bidirectionally along the strand from isolated points of origin. The protoxylem tracheids, before their elongation, have no specific pattern of secondary wall deposition. The type of wall-thickening may vary from spiral to reticulate. Later-formed xylem has bordered pits.

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PURIFICATION AND CRYSTALLIZATION OF SOUTHERN BEAN MOSAIC VIRUS [1]

W. C. Price

A NEW virus disease of garden bean (*Phaseolus vulgaris* L.) was discovered by Zaumeyer and Harter in 1942. Because the disease was found in the southern states, they called it southern bean mosaic. Later, they proposed the binomial *Marmor laesiofaciens* for the pathogen (Zaumeyer and Harter, 1943a).

Southern bean mosaic (SBM) virus is unusually heat stable; it can be obtained in relatively large quantities from certain varieties of beans; it produces, in other varieties, necrotic primary lesions which provide a means of measuring its activity (Zaumeyer and Harter, 1943a). These facts suggested that the virus might be purified by the methods commonly employed for the purification of proteins and that it might provide suitable material for investigations of the fundamental nature and properties of viruses. It was obtained from Dr. Zaumeyer in the fall of 1943 and studies of its properties and methods for its purification were soon begun. These studies led to the isolation of the virus in crystalline form, as was outlined briefly in a recent paper (Price, 1945a).

In the course of the studies it seemed desirable, as an aid in the purification process, to investigate further some of the properties of the virus, and to determine the accuracy with which its activity can be measured by the local lesion method. Results of studies on pH stability, thermostability and accuracy of measurement of concentration will be published elsewhere (Price, 1945b, 1945c), as will the results of studies of physical properties. In the present paper it is proposed to describe in detail the purification and crystallization of the virus and to discuss some of its characteristics.

THE VIRUS.—Southern bean mosaic virus is not, so far as known, very widespread. Zaumeyer and Harter (1943a) obtained evidence which indicates that it occurs in Louisiana, Maryland, Mississippi, California, Colorado, and Idaho. It appears to be confined to the bean (*Phaseolus vulgaris* L.) and a few other species. Over 80 tested varieties of garden bean have proved susceptible; some develop necrotic primary lesions, others show systemic

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mottling symptoms, while a strain of the Blue Lake variety develops a systemic necrosis (Zaumeyer and Harter, 1944). Inheritance of symptom expression is controlled by a single genetic factor, the local-lesion response being dominant to the systemic-mottle response (Zaumeyer and Harter, 1943b). In many varieties, such as Bountiful, systemic symptoms are mild, thus making it difficult to distinguish infected plants from healthy ones. Two different strains of SBM virus have been differentiated; these differ somewhat in symptom expression in mottling-type host plants but have essentially the same properties (Zaumeyer and Harter, 1943b). The virus is readily transmitted from diseased to healthy plants of susceptible species by the rubbing method of inoculation (Zaumeyer and Harter, 1943a). Whether it is transmitted by means of insects is not known. The virus in expressed juice of diseased bean plants has a ten-minute thermal inactivation point of about 95°C., is infectious at a dilution of 1:500,000, and withstands treatment for 30 minutes with strong alcohol, nitric acid and formaldehyde solutions (Zaumeyer and Harter, 1943a). It has been differentiated from ordinary bean mosaic virus not only by its properties but also by means of cross protection tests (Zaumeyer and Harter, 1943a).

DIFFERENTIATION FROM OTHER VIRUSES.—The properties of SBM virus indicated that it was not closely related to any other well-known plant virus. However, before proceeding with purification it seemed desirable to bring additional evidence to bear on this point, for if SBM virus were merely a strain of a virus that had already been purified and studied in detail, its purification would not have nearly so much interest as if it were distinct from other viruses that have been purified. Plant viruses are generally differentiated and classified on the basis of their symptomatology, means of transmission, properties, serological reactions, immunological reactions and host range. The fact that SBM virus has a high thermal inactivation point seemed to differentiate it from most other plant viruses, for only two others, tobacco mosaic and tobacco necrosis viruses (*Marmor tabaci* H. and *M. lethale* H.), are known to withstand heating for ten minutes at 90°C. (Price, 1933, 1938). It can be differentiated from these two viruses on the basis of its host range and immunological properties.

HOST RANGE.—Zaumeyer and Harter (1943a) tested 35 species of plants in five different families for susceptibility. The three species, soybean (*Glycine max* Merr.), lima bean (*Phaseolus lunatus* L.), and bean (*P. vulgaris* L.) in the Leguminosae were found to take the virus. One species in the Chenopodiaceae, one in the Cruciferae, one in the Cucurbitaceae, 23 in the Leguminosae and six in the Solanaceae were found insusceptible.

In the present work, 21 species in twelve different families were tested by rubbing two or more leaves on one or more plants of each species with a cheesecloth pad saturated with the virus. After a suitable interval, varying from 7 to 20 days, the inoculated plants were observed for symptoms. Whether showing symptoms or not, they were tested for the presence of SBM virus by subinoculation from inoculated and newly formed, uninoculated, leaves to Early Golden Cluster bean plants.

Infection was obtained in soybean, lima bean and bean, and virus was recovered from these species, thus confirming the results of Zaumeyer and Harter. The other species tested appeared to be refractory to infection as no symptoms were produced in them and no virus could be recovered. Species which failed to become infected were: ACANTHACEAE—*Thunbergia alata* Bojer. EUPHORBIACEAE—*Euphorbia heterophylla* L. LEGUMINOSAE—*Crotalaria retusa* L.; *Lathyrus latifolius* L.; *Lespedeza striata* Hook. and Arn.; *Lupinus luteus* L.; *Trifolium agrarium* L.; *Vicia villosa* Roth. MALVACEAE—*Hibiscus manihot* L. NYCTAGINACEAE—*Mirabilis jalapa* L. OXALIDACEAE—*Xanthoxalis stricta* (L.) Small. PAPAVERACEAE—*Eschscholtizia californica* Cham. POLEMONIACEAE—*Phlox drummondii* Hook. RANUNCULACEAE—*Delphinium cultorum* Voss.; *Aquilegia caerulea* James. SCROPHULARIACEAE—*Nemesia strumosa* Benth. TROPAEOLACEAE—*Tropaeolum majus* L. VIOLACEAE—*Viola odorata* L.

Two of these insusceptible species, *Lupinus luteus* and *Vicia villosa,* had previously been tested by Zaumeyer and Harter; the others had not. Therefore, the present tests combined with those of Zaumeyer and Harter have shown only three species of plants, all in the Leguminosae, to be susceptible and 27 species in the Leguminosae and 21 species in 15 other families to be insusceptible. This limited host range suggests that the virus is not closely related to tobacco mosaic or tobacco necrosis viruses, both of which are capable of infecting experimentally inoculated plants in many families, nor indeed to tomato bushy stunt virus (*M. dodecahedron* H.) which it resembles in some other respects. The evidence thus obtained, however, is far from conclusive since host range is not a reliable index of relationship. For example, cucumber viruses 3 and 4, which are known to be related to tobacco mosaic virus, are much more restricted in host range than some other strains of this virus (Bawden and Pirie, 1937).

CROSS PROTECTION EXPERIMENTS.—It is well known from the work of Thung (1931), Salaman (1933), Kunkel (1934) and others that plants thoroughly invaded by one strain of a virus are refractory to infection with other strains of the same virus while remaining at the same time susceptible to infection with unrelated viruses. This fact was utilized by Zaumeyer and Harter (1943a) in testing for a relationship between SBM and common bean mosaic viruses. They found that Low Champion bean plants diseased with ordinary bean mosaic

virus, as a result of seed transmission, were fully susceptible to necrotic-type infection by SBM virus. Thus they were led to conclude that these two viruses are unrelated.

The Scotia variety of bean develops systemic mottling symptoms when inoculated with SBM virus, but it responds to infection with tobacco necrosis and tobacco mosaic viruses by the production of characteristic necrotic local lesions. It was, therefore, a favorable host plant to use in experiments involving these viruses. Since alfalfa mosaic virus (*Marmor medicaginis* H.) is another that produces necrotic primary lesions in Scotia bean plants, it was included in the tests. The experiments were carried out by inoculating a set of bean plants with SBM virus, waiting about ten days for systemic symptoms to develop fully, and then rubbing the first trifoliate leaf on each plant with one or another of the viruses to be tested. The controls consisted of an equal number of Scotia bean plants, held under the same conditions, but not given the preliminary inoculation with SBM virus. Tests with tobacco necrosis virus were carried out under ordinary greenhouse conditions. However, since high temperature and low light intensity are more favorable for production of tobacco mosaic virus lesions, tests with this virus were conducted by holding the plants in the dark at a temperature of 90–100°F. for a day previous to inoculation and for three days after inoculation with tobacco mosaic virus. Tests with alfalfa mosaic virus were likewise made at the high temperature. Results were clear cut. They are illustrated in figure 1. All the leaves inoculated with tobacco necrosis, tobacco mosaic, and alfalfa mosaic viruses developed numerous lesions irrespective of whether they had previously been infected with SBM virus or not. The experiments showed that SBM virus did not protect bean plants from infection with any one of these three viruses, and thus brought evidence that it is not closely related to any of them.

Most of the viruses that attack beans and other leguminous plants, such as pea mosaic, pea mottle, and red clover vein mosaic viruses (*Marmor leguminosarum* H., *M. efficiens* Johnson, and *M. trifolii* H.), have properties in sharp contradistinction to those of SBM virus. Most of them are inactivated by temperatures far lower than that required for inactivation of SBM virus, and they withstand aging *in vitro* for only relatively short periods of time. Pea streak virus of Chamberlain (1939) seems to be different in this respect, however, since it is reported to withstand aging for 41 days or more and to require heating for ten minutes at 78–80°C. to inactivate it. Moreover, it produces necrotic local lesions on certain varieties of beans. On the other hand it appears to have a much broader host range than SBM virus, being infectious for three species in the Cucurbitaceae and for 17 species in the Leguminosae. Unfortunately, this virus was not available to the writer and, therefore, a direct comparison between it and SBM virus could not be made.

In view of the results reported above it would appear that SBM virus belongs to a new immunological group and that it is not closely related to any of the previously described viruses of plants, with the possible, though not probable, exception of Chamberlain's pea streak virus.

PURIFICATION.—The virus used in purification procedures was obtained from plants of the Bountiful variety of bean, grown in four-inch pots in a

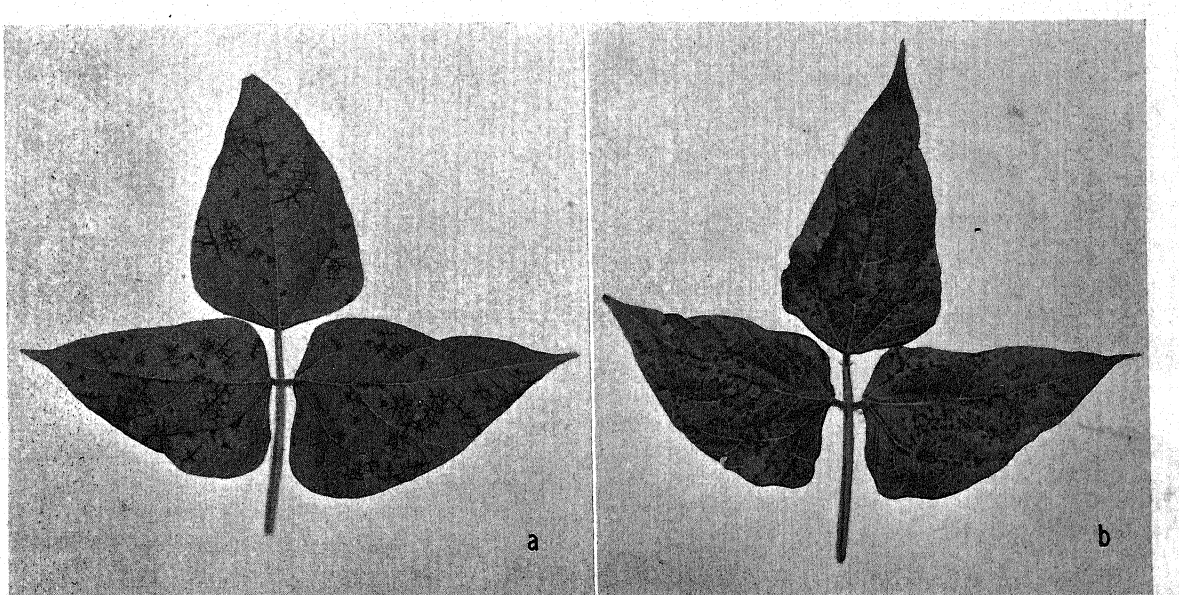


Fig. 1. Trifoliate leaves from Scotia bean plants infected first with southern bean mosaic virus and subsequently with (a) tobacco necrosis virus or (b) tobacco mosaic virus. The numerous necrotic lesions show that SBM virus did not protect from infection with either of the other viruses.

greenhouse. The Bountiful variety is a bush type, is easily infected, and develops only mild systemic symptoms (fig. 2). The plants were inoculated, by the rubbing method, when the primary leaves were about two-thirds expanded and were harvested about three weeks later. They were frozen at —10°C. and ground in a power food grinder when needed. Juice was extracted from the resultant pulp with pressure by hand through a single thickness of cheesecloth. A second extraction of the pulp was made with about a half volume of four or five per cent Na_2HPO_4 or K_2HPO_4. This second extraction served a useful purpose in removing much extraneous material from the juice, for a greenish flocculent precipitate developed when the two extracts were mixed, and the precipitate was removed by centrifuging at 3000 r.p.m. for a few minutes. It also increased the yield of virus, but by a varying amount. In one experiment, for example, less than ten per cent, in another nearly 100 per cent, of the amount of virus originally present in juice was obtained by subsequent extraction of the pulp.

Purification of the virus was carried out at room temperature by means of chemical fractionation, by alternating cycles of high and low speed centrifugation, or by a combination of the two methods. For chemical fractionation, the virus was precipitated once or twice with $(NH_4)_2SO_4$, and the precipitate removed by low speed centrifugation, then dissolved in an amount of water equal to about one-tenth the volume of the starting material. The quantity of $(NH_4)_2SO_4$ required for precipitation of the virus varied with the virus concentration and the temperature, about 300 gms./liter of starting material being required for the first precipitation and a somewhat smaller amount for the later ones. After the virus had been partially purified by treatment with $(NH_4)_2SO_4$, a sufficient volume of 95 per cent alcohol to give a 30 per cent solution was added and the resultant precipitate removed by low speed centrifugation. The precipitate consisted mainly of water-insoluble material and possessed little or no virus activity. To the supernatant were added two volumes of water in order to reduce the alcohol concentration to a point where sufficient $(NH_4)_2SO_4$ would go into solution to precipitate the virus. The $(NH_4)_2SO_4$ precipitate was removed by centrifugation and taken up in water. The virus was further concentrated and purified by precipitating once or twice more with $(NH_4)_2SO_4$ or $MgSO_4$.

In purifying the virus by high speed centrifugation, the starting material was centrifuged, in the early experiments, for 2½ to 3 hours in a Bauer and Pickels (1936) type centrifuge at 24,000 r.p.m. or, in later experiments when large volumes of material were used, in a Sharples high speed centrifuge at the rate of 10 cc./min. The yield of virus appeared to be about the same for the two procedures. The sediment was taken up in a volume of water equal to about one-tenth to one-hundredth that of the starting material and the solution thus obtained was centrifuged at 3000 r.p.m. for ten or 15 minutes to remove aggregated colloidal material. The solution was subjected to one or more additional cycles of high and low speed centrifugation. In aqueous solutions the pellets failed to pack

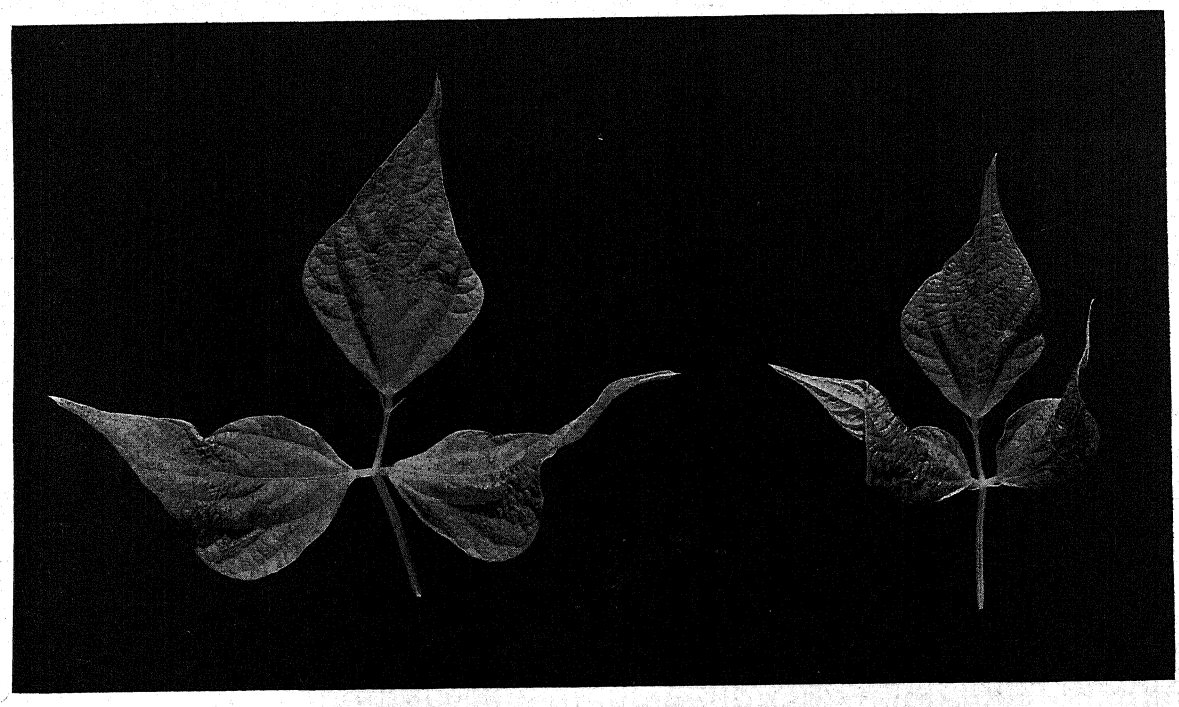


Fig. 2. Systemic symptoms of SBM virus in trifoliate leaves of Bountiful bean plants. The photograph was taken about three weeks after the plants were inoculated.

TABLE 1. *Relative activities of fractions obtained in various experiments during process of purification by chemical fractionation of southern bean mosaic virus.*

Treatment	Relative activity[a]	Relative volume[a]	Virus recovered
	per cent		per cent
First $(NH_4)_2SO_4$ precipitate	632	1/7.5	85
	566	1/7.0	80.9 [40.3–160][b]
	847	1/11.7	72.5 [58.4–90.6]
First $(NH_4)_2SO_4$ supernatant	1.6	1/1	1.6
	6.6	1/1	6.6 [2.1–21.3]
Fraction soluble in alcohol	312	1/4.2	74.9 [46.9–110]
	135	1/10	13.5 [5.0–18.6]
	158	1/10	15.8 [7.3–33.0]
	912	1/25	36.4
Fraction insoluble in alcohol	35.5	1/37.5	0.95
Final purified product	3700	1/136	27.2 [15.5–47.7]
	4020	1/300	13.4 [9.4–19.1]
	2280	1/300	7.6 [6.8–8.5]

[a] As compared with the starting material.
[b] Figures in brackets represent range as calculated from the standard deviation of the estimate at the 0.05 level of significance.

tightly even after three hours at 24,000 r.p.m., but this was overcome by adding enough $MgSO_4$ to give about a 0.1 per cent solution, before each centrifugation.

During the course of the purification procedures, the activities of various fractions were determined by inoculating Early Golden Cluster bean plants with appropriate dilutions of the samples. In most cases, the method described elsewhere (Price, 1945c) was followed. Typical activity measurements are summarized in tables 1 and 2. The data show that the purification procedures resulted in considerable loss of virus, the yield varying from about 8 to about 56 per cent of the virus originally present. Since the fractions that were discarded had relatively little virus activity, the loss was probably due to inactivation. In one instance centrifugation at 24,000 r.p.m. was allowed to proceed for only one hour, in which case the supernatant contained more virus than the pellets.

The virus was isolated from 16 lots of bean plants grown at various seasons of the year in a greenhouse. The appearance of purified preparations thus obtained varied with the source of the starting material. Some lots of material gave preparations that were light brown in color and on centrifugation at high speed yielded large amber colored pellets. Other lots resulted in dark, almost black, solutions that yielded black pellets on high speed centrifugation. Repeated centrifugation of such preparations failed to remove the color, which was apparently due to small amounts of pigment adsorbed on the virus particles, nor could the pigment be removed by precipitating the virus with $(NH_4)_2SO_4$. The presence or absence of the pigment appeared to be correlated with the light and temperature conditions under which the source plants were grown; plants grown at moderately high temperatures in summer produced large amounts of pigment, while those grown at somewhat lower temperatures in

TABLE 2. *Relative activities of fractions obtained in various experiments during process of purification by high speed centrifugation of southern bean mosaic virus.*

Treatment	Relative activity[a]	Relative volume[a]	Virus recovered
	per cent		per cent
First high speed centrifugation	14,900	1/200	74.5 [57.5–97.5][c]
Supernatant from first high speed centrifugation	6.3	1/1	6.3
Second high speed centrifugation	7,950	1/143	55.6
	2,820	1/167	16.9
	5,970	1/600	10.0 [7.1–14.0][b]
Supernatant from second high speed centrifugation	31.6	1/7.4	2.3
	12.6	1/13.4	0.6
	4,730	1/200	23.6 [13.4–43.6][b]

[a] As compared with the starting material.
[b] After only 1 hour at 24,000 r.p.m.
[c] Figures in brackets represent the range as calculated from the standard deviation of the estimate at the 0.05 level of significance.

TABLE 3. *Activity of purified preparation containing 10^{-3} gms./cc. of southern bean mosaic virus at various intervals after freezing at —10°C. as compared with an aliquot held at 3°C.*

Time frozen	Activity Most probable	Range[a]
	per cent	per cent
6 days	5.0	3.4–7.3
11 days	1.6	1.1–2.2
3 months	0.0	.....

[a] As calculated from the standard deviation of the estimate at the 0.05 level of significance.

winter produced less pigment. Some of the pigment could be removed by treating dark colored solutions with a small amount of activated charcoal (norite) and filtration through celite on a Büchner funnel. This procedure was attended, however, by some loss in virus activity. The presence of pigment interfered with crystallization of the virus, for only preparations relatively free of pigment crystallized readily by the method to be described later.

Highly purified preparations of the virus gave positive Biuret and xanthoproteic tests for protein and negative Molisch's and Fehling's tests for carbohydrate. With Millon's reagent, solutions containing about 1 mg./cc. formed a precipitate which failed to turn red on heating, suggesting a low tyrosine content. The glyoxylic reaction, a test for tryptophane, was negative with dilute virus solutions but positive with solutions containing from 5 to 10 mg./cc. of virus.

As will be shown elsewhere[2], purified preparations appeared to be essentially homogeneous when studied in an electrophoresis apparatus, a high speed centrifuge and a diffusion apparatus. It would thus appear that such preparations were essentially free of impurities. They did not show stream double refraction when flowing under crossed polaroid plates. This suggested that the virus particles have low geometrical asymmetry.

The yield of virus, on a protein nitrogen basis, from infected Bountiful bean plants varied from 0.2 to 0.4 mg./cc. of the original juice. Since activity measurements indicated that a considerable fraction, perhaps half or more, of the virus was usually lost in the purification process, it is probable that the juice of infected plants contained from 0.4 to 0.8 mg./cc. of virus that could be extracted by the procedures employed.

EFFECT OF FREEZING.—Purified preparations of SBM virus remain active for months when stored at 3°C. However, they rapidly become inactive when frozen. In one test, for instance, a purified sample containing 10^{-3} gms./cc. of virus was divided into two lots; one lot was frozen at —10°C. and the other lot held at 3°C. as a control. Tests for activity were made at various intervals by the method previously described, with the results shown in table 3. In this experiment, the frozen virus lost about 95 per cent of its activity in six days and about 98.4 per cent in eleven days and apparently was completely inactive after three months.

CRYSTALLIZATION.—Southern bean mosaic virus was crystallized from highly purified preparations containing 10 mg./cc. or more virus in the following manner. The preparations were brought to about 20 per cent saturation with respect to $MgSO_4$ or $(NH_4)_2SO_4$ and centrifuged for 2½ to 3 hours at 24,000 r.p.m. The virus was packed tightly in the form of amber colored pellets at the bottom of the centrifuge tubes. The supernatant was poured off and discarded. One or two drops of water were added to each pellet which was allowed to stand for several hours at 3°C. The pellets gradually became opaque and were later found to contain masses of



Fig. 3. Crystalline southern bean mosaic virus. The photograph represents a rather thick preparation of the rhombic prisms described in the text. Magnification about 350 diameters.

crystals. The saturated solution in which the crystals had formed was removed and two drops of water again added to the remainder of each pellet. As this procedure was continued, all the virus was eventually removed from the centrifuge tubes. It was found to consist partly of crystals and partly of

[2] Data to be published.

virus in solution, the latter making up from 80 to 90 per cent of the total.

The crystals were of one of two types. That illustrated in figure 3 may be described as a rhombic prism; that in figure 4 as a rhombic bipyramid joined by two pinacoids. Although it has not been possible to measure their interfacial angles, it is concluded tentatively, from careful microscopic examination, that both types belong in the orthorhombic system. This conclusion was reached with some hesitation for neither type of crystal has been observed to show birefringence when examined under a polarizing microscope. It is possible that they are weakly birefringent and, being small, do not show this phenomenon with sufficient brilliancy to be detected with the equipment at hand.

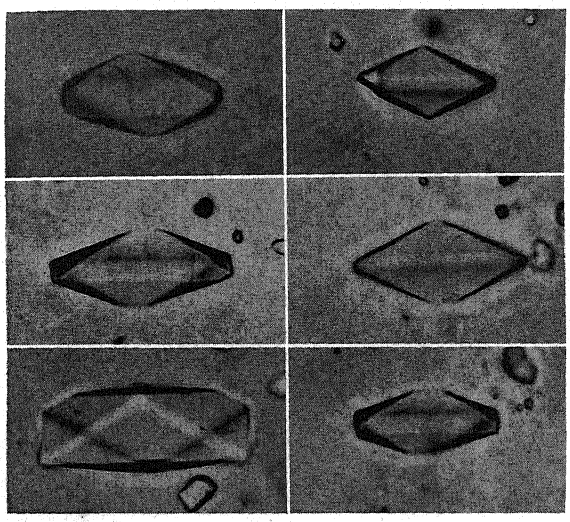


Fig. 4. Crystalline southern bean mosaic virus. A composite photograph showing isolated crystals of the type referred to as a rhombic bipyramid joined by two pinacoids. In preparing the specimens, not all the amorphous material was removed, and some of it can be seen in the photographs. Magnification about 790 diameters.

The fact that two different types of crystals were obtained from the virus is not surprising, for many substances are known to give different crystalline forms depending upon the method of crystallization. In the present case it was not possible to tell ahead of time which crystalline form would be obtained. In fact, in one experiment, the first crystals to come off were rhombic prisms and, when these were removed, what was left of the pellets gave off bipyramids when more water was added.

The crystals were freed of virus in solution by repeatedly washing them rapidly with 25 per cent saturated $MgSO_4$. The washed crystals were stored in half-saturated $(NH_4)_2SO_4$. They were readily soluble in water containing only a trace of salt and aqueous solutions of them were highly active.

Solutions of the crystals have shown, insofar as tested, properties identical with those of the mother liquor. They gave the usual tests for proteins and negative tests for carbohydrates. They produced typical symptoms of SBM virus when used to inoculate Bountiful beans.

Representative tests of their activities as compared with those of their mother liquors are summarized in table 4. These data showed no significant differences between the activity of the bipyramids and the prisms nor between these and the mother liquors from each, when compared on a weight basis. The minor differences observed at the various dilutions are ascribed to chance variation.

The rhombic prisms of southern bean mosaic virus are similar in appearance to those of tobacco necrosis (Pirie *et al.*, 1938) and tomato bushy stunt (Cohen, 1942) viruses. The bipyramids, on the other hand, appear to be distinct from the crystal forms of any of the other viruses that have been crystallized. The fact that all three of these viruses crystallize in the form of thin rhombic prisms may or may not have significance in respect to their relationships. It has definitely been shown that none of the three are closely related, but each has spherical particles whose sizes are of the same order, as will be shown in the section on electron micrography.

Attempts to crystallize SBM virus by the methods used successfully in the case of tobacco mosaic (Stanley, 1935, 1936), tomato bushy stunt (Bawden and Pirie, 1938a, 1938b), and some isolates of tobacco necrosis (Pirie *et al.*, 1938; Bawden and Pirie, 1942) viruses resulted in failure. When $(NH_4)_2SO_4$ was added to highly purified solutions

TABLE 4. *Activity of the bipyramidal crystals (A), their mother liquor (B), the rhombic prisms (C), and their mother liquor (D) as indicated by the numbers of lesions per leaf produced on 24 leaves of Early Golden Cluster beans.*

Log concentration	Lesions per leaf							
	Test 1				Test 2			
	A	B	C	D	A	B	C	D
gms./cc.								
—4	169.2	107.5	139.3	107.0	...	...	...	...
—5	56.8	33.8	45.8	28.9	7.8	28.2	37.8	19.1
—6	6.2	9.6	9.9	2.8	10.6	7.6	2.8	2.3
—7	1.4	1.3	0.6	0.3	1.2	2.7	0.5	0.3
—8	1.1	0.4	0.5	0.1	0	0.2	0	0

at room temperature or in the cold, either as the solid salt or as a saturated solution dropwise, an amorphous precipitate was invariably obtained. When the salt was added at room temperature until a precipitate was obtained, and the virus preparation then removed to 3°C., the precipitate went into solution in from 30 minutes to one hour and remained in solution when held for as long as a week at this temperature. When it was warmed up slowly to room temperature, an amorphous precipitate was again obtained.

FAILURE TO ISOLATE SIMILAR MATERIAL FROM HEALTHY PLANTS.—The juices of healthy Bountiful bean plants have been subjected to the treatments used to isolate SBM virus from diseased plants but no heavy weight material resembling the virus has been isolated from them. It is known that there is present in the juice of healthy plants of certain species normal particles roughly the size of certain viruses (Loring *et al.*, 1938; Price and Wyckoff, 1938, 1939). These materials are easily destroyed in the presence of strong salt solutions or on exposure to moderately high temperatures or other unfavorable environmental conditions. If such materials were present in the juices of Bountiful bean plants, they were not isolated by the procedures employed for the isolation of the virus.

ELECTRON MICROSCOPE PHOTOGRAPH OF SOUTHERN BEAN MOSAIC VIRUS.—Figure 5 shows a photograph of a purified preparation of SBM virus taken with an RCA model EMC-1 electron microscope. The sample that was photographed contained 10^{-4} gms./cc. of virus. It is evident from this and other photographs that the virus preparation was highly uniform with respect to size and shape of the particles it contained. This agrees with the data obtained when the same preparation was analyzed in a high speed centrifuge, an electrophoresis apparatus and a diffusion apparatus. It is also evident that the particles are essentially spherical, thus confirming the results obtained when the virus was tested for stream double refraction. Measurements of the images give an average value of 33.6 mμ for the diameter of the particles. This value is not considered particularly accurate in view of the fuzziness of the boundaries in the photograph, the small size of the particles and the possible error involved in the calibration of the microscope. However, considering these factors, the value agrees well with that obtained for the diameter of the particles from sedimentation and diffusion data.[3]

DISCUSSION.—Southern bean mosaic virus is the fourth to be crystallized; the other three, including some of their various strains, are tobacco mosaic (Stanley, 1935, 1936), tomato bushy stunt (Bawden and Pirie, 1938a, 1938b), and tobacco necrosis (Pirie *et al.*, 1938) viruses. The method of obtaining crystals of SBM virus was somewhat different from those hitherto used. It may, perhaps, prove of value in crystallizing some of the viruses that already have been isolated in essentially pure form but have not responded to other methods, or some that may be isolated in the future. It is of significance that all four viruses that have been crystallized are relatively stable; three of them have thermal inactivation points above 90°C. (Price, 1933, 1938; Zaumeyer and Harter, 1943a) and the other about 80°C. (Smith, 1935); all four may be held at room temperature for relatively long periods without appreciable loss of activity and at refrigerator temperatures for even longer periods. Paralleling this high degree of heat stability is the ability to withstand the relatively rough treatments applied in the purification and crystallization processes.

It was the high thermal inactivation point of SBM virus that led the writer to attempt its crystallization, although at the time the work was planned it was not known whether the virus could be obtained readily in the quantities needed or whether its particles were of such a shape that they could

[3] These data are to be published later.

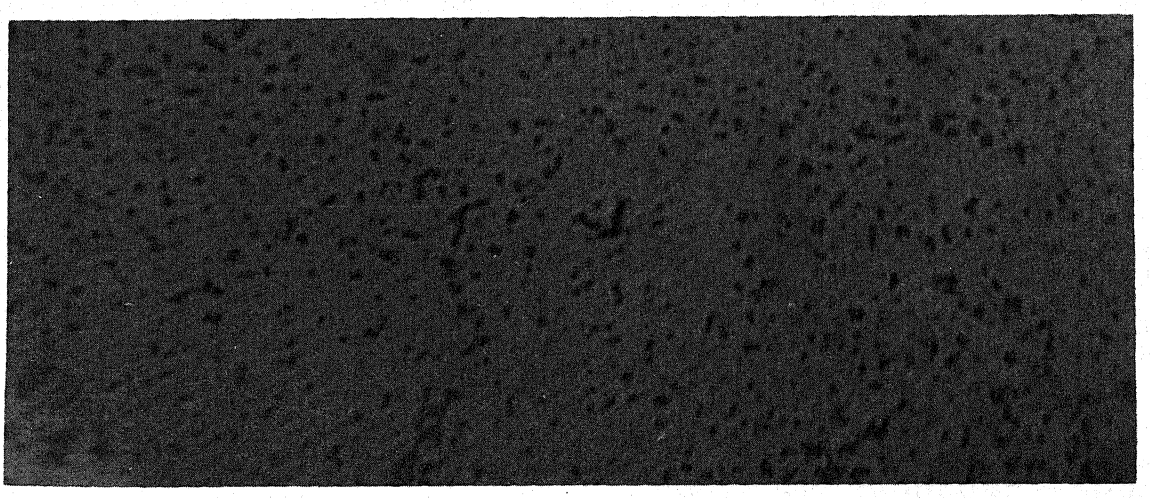


Fig. 5. The spherical particles of southern bean mosaic virus as seen under an electron microscope. Magnification about 31,500 diameters.

form crystals. Such regularity of arrangement as is necessary for crystallization might be difficult for particles of certain shapes, such as, for example, the sperm-shaped particles of some bacteriophages, which electron microscopy has revealed as consisting of a relatively large head and a long thin tail (Luria and Anderson, 1942; Luria, Delbrück, and Anderson, 1943). Tobacco mosaic virus particles, while uniform in thickness, vary considerably in length (Stanley and Anderson, 1941), and this may be why they orient in two directions to form paracrystals rather than in three directions to form true crystals. The spherical particles of SBM virus, being of uniform size, are readily arranged in three-dimensional regularity.

Crystallization of southern bean mosaic virus is not proof of its purity; it is merely one of several observations which indicate that the virus has been obtained in relatively pure form. Data from high speed centrifugation, electrophoresis and diffusion experiments, which will be published elsewhere, indicate that the virus preparations studied were essentially homogeneous and thus substantiate the evidence of crystallization for the purity of the samples. This evidence is also substantiated by electron micrography which shows the particles as essentially homogeneous with respect to size and shape. Chemical tests that have been applied suggest that the particles are composed mainly of nucleoprotein.

The fact that certain viruses can be induced to crystallize has been considered by many workers as evidence of their non-living nature. However, since crystallinity is merely a positional regularity, it cannot be considered theoretically impossible for living organisms to orient in three-dimensional regularity and form true crystals. This possibility was suggested as early as 1938 by Andrewes. Whether viruses in general, and SBM virus in particular, are chemical molecules, or living organisms is at present a matter of conjecture. Recently Wyckoff (1945) concluded that there have been no experimental data bearing directly on the question. It seems not unreasonable, however, that its solution is within grasp and that it may yield to experimentation within the next few years.

It will be instructive to subject purified preparations of SBM virus to physical and chemical measurements such as have already been applied to the other "stable" viruses. X-ray and electron diffraction patterns of the crystals, absorption spectra and antigenicity of the virus particles are only a few of the types of investigations that may prove fruitful. When more material is available, it should be possible to study the amino acid composition of the virus and some of its other chemical constituents. The differences between viruses are likely to prove as illuminating as their similarities with respect to their fundamental nature.

SUMMARY

This paper describes in detail the purification and crystallization of southern bean mosaic virus (*Marmor laesiofaciens* Zaumeyer and Harter). Southern bean mosaic virus is differentiated from other plant viruses on the basis of its high degree of thermostability, the symptomatology of the disease it produces and its host range. Cross protection tests showed that it is not closely related to tobacco mosaic, tobacco necrosis, or alfalfa mosaic viruses, although it resembles each of these in some respects.

The virus was concentrated and purified either by means of alternating cycles of high and low speed centrifugation or by chemical fractionation, with essentially identical end products. Purified virus appeared homogeneous when examined in an analytical centrifuge, an electrophoresis apparatus, a diffusion apparatus and an electron microscope. Such preparations gave positive tests for protein and negative tests for carbohydrate and were found to contain spherical particles having a mean diameter of about 33.6 mμ. They remained active for months when held at about 3°C. but soon became non-infectious when frozen.

Crystallization was carried out by centrifuging purified preparations, brought to about 20 per cent saturation with $MgSO_4$ or $(NH_4)_2SO_4$, at 24,000 r.p.m. to obtain amber colored pellets, draining off the supernatant liquid, then adding only one or two drops of water and allowing to stand for several hours at 3°C. The crystals were either rhombic bipyramids joined by two pinacoids or rhombic prisms, both probably belonging to the orthorhombic system though neither showed birefringence under a polarizing microscope. Solutions of the crystals were highly active and produced typical symptoms of southern bean mosaic.

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DOUBLE FERTILIZATION IN PETUNIA [1]

D. C. Cooper

AN UNEXPECTED variation from the general course of double fertilization has been described as occurring in *Petunia nyctaginiflora* by Ferguson (1927). She found that, when the pollen tube reaches the egg apparatus, and prior to fertilization, the endosperm mother cell undergoes a series of divisions to form a four-celled diploid endosperm. Double fertilization then occurs, one sperm nucleus fusing with that of the egg and the other with that of the micropylar endosperm cell. As a result of fertilization the entire endosperm is stimulated to rapid development so that approximately one-fourth of the growing endosperm, that derived from the micropylar cell, is triploid (21 chromosomes) and the remainder is diploid (14 chromosomes). Since such a deviation has not been described as occurring in other members of the Solanaceae, the course of double fertilization in two cultivated varieties of *Petunia* was checked in order to verify this report. No similar divergence from the normal could be detected in this material.

Ovaries were collected at the open flower stage and at regular intervals thereafter from two varieties of the garden petunia, Elk's Pride and Topaz Queen, which were growing in the gardens of the Department of Horticulture, University of Wisconsin. They were fixed in Carnoy's solution for two to four hours, washed in 80 per cent alcohol and embedded in paraffin. Sections 12 to 15 μ in thickness were cut, mounted serially and stained in Delafield's haematoxylin.

The macrogametophyte at the open flower stage is a typical seven-celled structure (fig. 1). Only vestiges of the antipodal cells are present and these disintegrate shortly after fertilization. The egg apparatus at the opposite end of the gametophyte consists of three pear-shaped cells, the egg and two synergids. The egg has a large apical vacuole and its nucleus lies in the dense cytoplasm in the basal portion of the cell. The base of the egg extends into the gametophyte well beyond the synergids. A large basal vacuole is present in each synergid, and the small nucleus is embedded in the dense cytoplasm immediately above it.

The two polar nuclei lie in the dense cytoplasm of the endosperm mother cell closely adjacent to the egg, and a single large vacuole occupies the chalazal end of the cell. The polar nuclei usually fuse

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shortly after the gametophyte reaches maturity and the nucleoli may fuse so that one large nucleus with a single nucleolus is present at the time of fertilization. Occasionally this fusion does not take place and two nuclei are present at that time. Such fusion of the polar nuclei is common in the Solanaceae (Guignard, 1902; Ferguson, 1927; Glišič, 1928; Cooper, 1931; Kruger, 1932; Rees-Leonard, 1935). The dense cytoplasm of the endosperm mother cell immediately surrounding the fusion nucleus is packed with starch grains.

Fertilization occurs between 24 and 32 hours after the opening of the flower in both varieties. Pollen tubes are present in abundance on the placentae of ovaries collected at these intervals and those ovules in the apical portions of the ovaries collected at the first interval show stages of fertilization. Approximately all the ovules had been fertilized in material collected at the latter interval.

The two male gametes occupy a position near the tip of the pollen tube which enters the macrogametophyte between the apices of the two synergids and egg. The apical portion of the pollen tube enlarges and becomes somewhat flattened against the side of the egg. One male nucleus enters the egg and becomes closely associated with its nucleus, and that of the second male gamete enters the endosperm mother cell and becomes associated with the fused polar nuclei (fig. 2). Two cone-like projections are present at the apex of the pollen tube, one of which extends into the endosperm mother cell and the tip of the other is closely appressed to the egg. Their position indicates that the male gamete nuclei probably enter the macrogametophyte by way of these projections. Two deeply staining bodies remain in the tip of the pollen tube, the so-called "x-bodies." It is suggested that these may be the cytoplasmic remnants of the male gametes. A careful examination of these bodies failed to reveal any evidence that they are nuclei or that nuclei are enclosed within them.

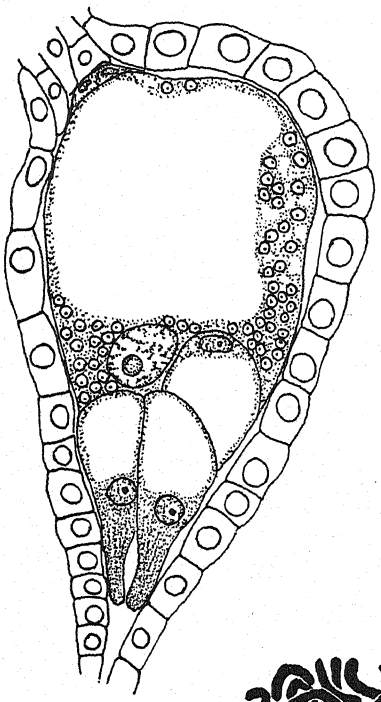
Shortly after fertilization the endosperm nucleus undergoes mitosis. The chromatic network of the male nucleus condenses well in advance of that of the fused polar nuclei. As a result seven short, well-developed chromosomes appear in one portion of the nucleus by the time the remainder of the nucleus contains 14 elongate chromosomes in a late prophase condition (fig. 3). Twenty-one chromosomes (3n) are present at the equatorial plate stage (fig. 4). The longitudinal axis of the spindle coincides with that of the primary endosperm cell. Nuclear division is followed by a transverse division of the primary endosperm cell (fig. 5), the micropylar cell being much smaller than the chalazal cell. Three nucleoli are present in each of the daughter nuclei at late telophase. These fuse to form a single large nucleolus as the nucleus approaches the resting condition. The micropylar cell divides almost immediately (fig. 6) to form a three-celled endosperm, and shortly thereafter the chalazal cell divides (fig. 8) so that a linear row of four cells is produced. The presence of 21 chromosomes on mitotic spindles in each of these cells (fig. 7, 9) shows definitely that all the cells of the four-celled endosperm are triploid. The tip of the pollen tube with its enclosed "x-bodies" disintegrates shortly after the endosperm has reached this stage of development. The starch stored in the primary endosperm cell is used up during the course of these divisions. Both longitudinal and transverse cell plates are formed during the further growth of the endosperm.

These observations clearly indicate that double fertilization in the cultivated *Petunia* takes place in the usual manner. Due to the great quantity of starch in the dense cytoplasm of the endosperm mother cell immediately adjacent to the fusion nucleus, the presence of the male gamete nucleus in close association with the fusion nucleus immediately following fertilization is difficult to detect. Many clear-cut stages such as that shown in figure 2 were, however, observed in both varieties demonstrating the fact that such fusion occurs. The presence of 21 chromosomes (3n) on the mitotic spindle of the first division of the endosperm fusion nucleus is conclusive evidence of the usual type of double fertilization. Six metaphase plates of this division have been observed whereon the chromosomes could be counted and in each instance this triploid number was present. In the one anaphase figure found, twenty-one chromosomes were passing to each pole.

The number of chromosomes on the second (fig. 7) and third mitotic (fig. 9) spindles leading to the four-celled endosperm stage has likewise been checked and in each case the triploid number was present. There is no evidence whatsoever that any of these cells are diploid as described by Miss Ferguson (1927). She shows indications of the occurrence of normal double fertilization in *P. nyctaginiflora* in her figure 4 of a late stage of the first division of the endosperm nucleus in that three nucleoli are present in one of the daughter nuclei and two are present in the egg nucleus. This is a stage similar to that shown in figure 5 of the present paper. As these daughter nuclei pass into a resting stage, the three nucleoli fuse to form one large nucleolus.

Redinger (1938), following intergeneric crosses, using *P. nyctaginiflora* as the pistillate parent, found that there was no fertilization by the foreign gametes. He did, however, find development of an occasional seed with a diploid embryo and triploid endosperm. Upon examination of early stages of development of such seeds, he discovered that union of the fusion nucleus with a nucleus which he describes as being that of one of the antipodals is necessary to bring about normal endosperm development. He shows a figure of the first division of the fusion nucleus with 21 chromosomes on the equatorial plate.

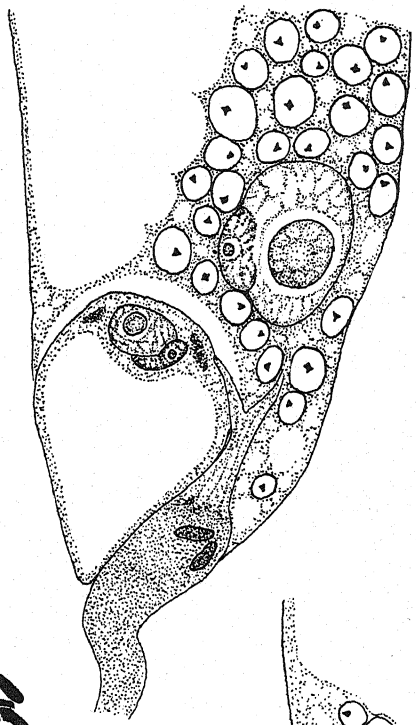
The two densely staining bodies in the tip of the pollen tube which Miss Ferguson describes as sperm



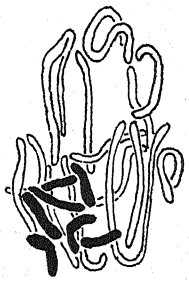
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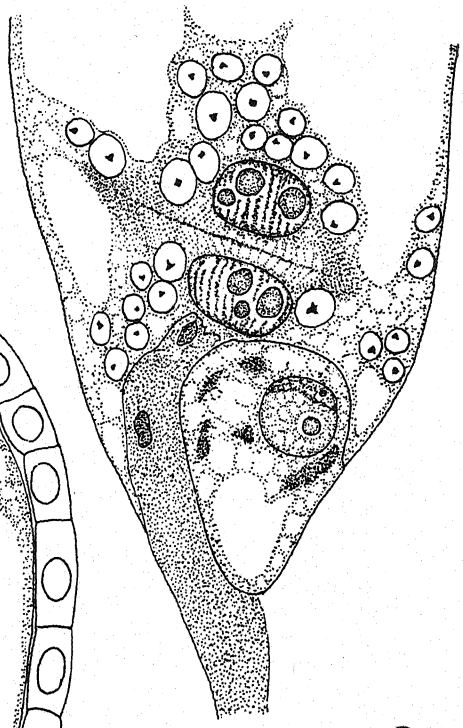
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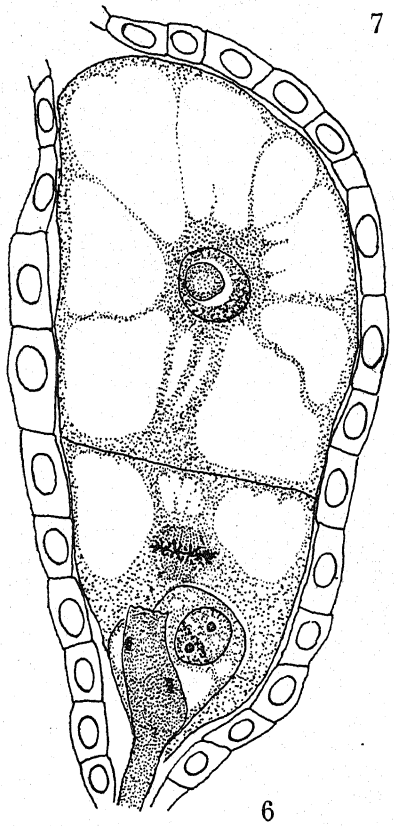
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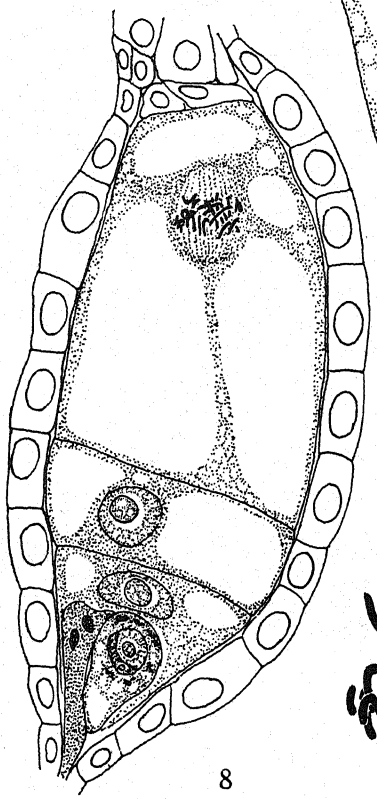
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nuclei are, in all probability, the "x-bodies" shown in figures 2, 5, 6 and 8. These bodies, as she found, show "little or no" structure. The remnants of the pollen tube and its contents disintegrate shortly after the four-celled endosperm stage is reached.

The writer is at a loss to account for the presence of the diploid number of chromosomes on a mitotic spindle in a chalazal endosperm cell at the 16-celled stage of development (Miss Ferguson's fig. 15). It is possible that an endodermal cell was mistaken for an endosperm cell. This adjacent maternal tissue is stimulated to active cell division as a result of fertilization and carries the diploid number of chromosomes. A dividing nucleus in the chalazal region of an endosperm of similar size was found in the present investigation, and twenty-one chromosomes were present on the equatorial plate (fig. 10).

When the number of chromosomes is doubled, the size of the nucleus increases and such change is very close to proportional, especially when one is careful to measure only those nuclei which are at interphase, the so-called "resting" stage. This being so, it was decided to determine if a similar size relationship exists between diploid and triploid nuclei. A series of such measurements was made in order to compare the size of the nuclei of the embryo with those at either end of the accompanying endosperm. It was found that, on the average, the volume of an embryo nucleus is approximately two-thirds that of an endosperm nucleus and that there is no significant difference in size between endosperm nuclei lying near the embryo and those in the chalazal region. This is further evidence that a mosaic type of endosperm such as was described by Miss Ferguson is not characteristic of the varieties of *Petunia* used in the present study.

SUMMARY

The mature embryo sac of the garden petunia is a seven-celled structure consisting of three shrunken antipodals, a three-celled egg apparatus and a large endosperm mother cell. The two polar nuclei usually fuse prior to fertilization.

Fertilization occurs in the apical ovules of the ovary approximately 24 hours after the open flower stage and the basal ones are being fertilized at 32 hours.

Double fertilization takes place in the usual manner and the entire endosperm is triploid from its inception.

Two "x-bodies" remain in the tip of the pollen tube following discharge of the male gamete nuclei.

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Fig. 1–10.—Fig. 1. Mature macrogametophyte and adjacent endothelium just prior to fertilization showing small antipodals, egg apparatus and large endosperm mother cell with its fused polar nuclei and abundance of stored starch. ×770.—Fig. 2. Apical portion of macrogametophyte showing double fertilization. Two "x-bodies" are present in the apex of the pollen tube. ×1500.—Fig. 3. Late prophase stage in the division of the fusion nucleus of the endosperm mother cell showing the seven short, well-developed chromosomes from the male gamete nucleus and the 14 elongate chromosomes of the fused polar nuclei. ×2850.—Fig. 4. Twenty-one chromosomes approaching the equatorial plate in the first division of the endosperm fusion nucleus. ×2850.—Fig. 5. Apex of macrogametophyte with zygote and late telophase of dividing endosperm fusion nucleus showing cell plate formation. Three nucleoli are present in each of the daughter nuclei. "X-bodies" persist in tip of pollen tube. ×1500.—Fig. 6. Two-celled endosperm. Mitotic spindle in micropylar cell. ×1500.—Fig. 7. Late prophase in the division of the micropylar daughter cell of the two-celled endosperm showing 3n condition. ×2850.—Fig. 8. Three-celled endosperm. Nucleus of the chalazal cell is in a stage of division. The nuclei of the zygote are fusing and the tip of the pollen tube is still present. ×1500.—Fig. 9. Detail of the 21 chromosomes on the mitotic spindle in endosperm cell shown in figure 8. ×2850.—Fig. 10. Polar view of equatorial plate with 21 chromosomes in the chalazal end of a 16-celled endosperm. ×2850.

THE THREE-DIMENSIONAL SHAPE OF BUBBLES IN FOAM—AN ANALYSIS OF THE RÔLE OF SURFACE FORCES IN THREE-DIMENSIONAL CELL SHAPE DETERMINATION [1]

Edwin B. Matzke

When the early plant anatomists first began to study the organization of tissues, they were impressed by the general similarity in appearance of cells to foam. For nearly three centuries this comparison has been reiterated in the literature. Only within the last twenty years, however, have careful and extensive studies of the three-dimensional shapes of cells been made. The form of bubbles in foam has been a topic of considerable speculation and cursory observation for some decades, though apparently no thorough investigation has ever been made. Merely to establish a resemblance in shape between bubbles in foam and cells in tissues, between the constituent parts of purely physical and of living systems, would hardly be an end in itself. However, if the same forces are operative, wholly or in part, in both systems—and they undoubtedly are—then a precise study of bubbles in foam should make it possible not merely to recognize similar forces at work, but, within limits, to evaluate the relative importance of such forces in plant and animal development. With these considerations in mind, the present study was undertaken.

Literature review.—From the time of Robert Hooke in 1665 down to the present day the similarity between cells and bubbles in foam has been noted. Even in his Micrographia, Hooke (1665) included an "Observation" (XVIII) on the "Schematisme or Texture of Cork, and the Cells and Pores of some other such frothy Bodies"; and again, in describing pith, he compared it with the central part of the shaft of a feather, and spoke of "congeries of very small bubbles." Nehemiah Grew (1682), who had studied plant tissues intensively, was similarly impressed, for he wrote "the Parenchyma of the Barque is much the same thing, as to its conformation, which the Froth of Beer or Eggs is." Contrary to the popular misconception, both these early investigators were aware that cells had contents. Hooke noted that the cells were "fill'd with juices," while Grew described them more quaintly as "receptacles" and "Cisterns of Liquor."

The older literature on the three-dimensional shapes of cells has been reviewed in scholarly fashion by Lewis (1923, 1933) and by Marvin (1939b). All through the nineteenth century, from its very beginning (Mirbel, 1802) almost to its close (Gray, 1887), there were repeated references to the three-dimensional shapes of cells, frequently with illustrations. Almost without exception, cells in undifferentiated tissues were described as dodecahedra, and more commonly as fundamentally rhombic dodecahedra. A few authors, however, including Bernhardi (1805) and more especially Duchartre (1867), conceived of cells as having 14 faces. These conclusions in the nineteenth century were based on observations of cells and tissues in section, and on subsequent deductions. None of them embodied the results of careful and precise studies. This whole subject was reviewed in analytical form by Thompson in 1917, who at that time favored the rhombic dodecahedron as the basic type for cells in tissues. Twenty-five years later, however (1942), he modified his view, because of additional data which had been presented.

It is rather striking that, with all the discussions of the three-dimensional shapes of essentially undifferentiated cells, not one careful study actually was made until that of Lewis in 1923; since that year Lewis has investigated a whole series of tissues (1923, 1925, 1928a, 1928b, 1930, 1935, 1943a, 1943b, 1944). In all his extensive and meticulous researches he has demonstrated that the figure which cells in undifferentiated tissues tend to achieve is a polyhedron with 14 faces, and he has consistently defended the tetrakaidecahedron of Lord Kelvin (1887), with 8 hexagonal and 6 square faces (or its approximation), as the typical form. Lewis has shown not only how seemingly irregular forms may be derived from Lord Kelvin's figure, but how cells as essentially dissimilar as those of elderberry pith, the stellate pith cells of *Juncus effusus*, and the tracheids of *Pinus strobus* may all be modifications of this same tetrakaidecahedron.

Within the past five years a considerable number of other very careful investigations have been carried on, to define the three-dimensional shapes of cells in tissues and the way in which those shapes are established. Marvin has reported in great detail on the pith of *Eupatorium purpureum* (1939b) and of *Eupatorium perfoliatum* (1944). Hulbary (1944) examined pith cells of *Ailanthus glandulosa*. Dodd (1944) studied the parenchymal cells in the carpel vesicles of *Citrus grandis*. Higinbotham (1942) investigated the undifferentiated cells in the petiole of *Angiopteris evecta* and showed how intercellular spaces affect the number of faces. Most recently the importance of air spaces in the developing cortical cells of *Anacharis densa* has been analyzed by Hulbary (1944), and the effect of these large spaces on the shape of the cortical cells has been demonstrated. All these investigators have published tables of data, which have been drawn upon below for purposes of comparison.

In addition to the actual forms of cells and the ways in which these originate from a developmental standpoint, the underlying forces responsible for these forms have been discussed, and to some extent

[1] Received for publication May 7, 1945.

analyzed in the literature. In 1916 Harper mentioned such factors as "mutual pressure, adhesion, surface tension and the inherited form tendencies of the cells" in the alga *Pediastrum*. Mutual pressure as a factor in form determination in cellular aggregates has been analyzed in detail by compressing lead shot (Marvin, 1939a; Matzke, 1939). It has been demonstrated conclusively that when lead balls of relatively uniform diameter are compressed so that interstices are eliminated, they are changed into polyhedra averaging very nearly 14 faces (14.17 for 624 central shot), and that when small and large shot are mixed in various proportions and then compressed, the small ones are changed into polyhedra with fewer than 14 faces, and the large ones into polyhedra having more than 14 faces. The 14-faceted figures show certain similarities to cells in tissues, but they also show certain dissimilarities.

In his most recent publication, Lewis (1944) strongly emphasized the "necessities of spherical geometry" in cell shape determination.

Experimental work on the analysis of soap films in relation to the general problem of cell form goes back well into the last century. Holman, as reported by Child (1880, 1881), blew a mass of bubbles between two glass plates and examined them with the naked eye. According to this account, when the bubbles produced are nearly uniform in size, "the polygons formed are six-sided," and "those of a different number of sides exist where there is a diversity in the size of the air masses." A brief summary of experiments on soap films by Errera was published in 1888—he had published on this subject previously—and the close approach of very young plant cell walls to liquid films was emphasized there. Somewhat similar conclusions had also been voiced by Berthold (1886).

The most beautiful of the older demonstrations showing similarity between soap bubbles and cells was that of Robert (1902). He gave a striking series of photographs of groups of bubbles which simulate precisely the 4, 8, 12, and 16 cell stages in the cleavage of the gastropod *Trochus*. That similar forces are operative in both instances seems very highly probable.

These older workers, including Berthold, Errera, Robert, and many others, all admit their indebtedness to Plateau (1873). In his classical researches Plateau showed how soap films meet at angles of 120°, how edges meet in soap films at angles of 109°28′16″," and how the films become adjusted in various types of frames. Plateau's experiments and conclusions have retained their major significance down to the present. They were directly responsible for the description by Lord Kelvin (1887, 1894) of the "plane-faced isotropic" or "orthic" tetrakaidecahedron, and of his "minimal" tetrakaidecahedron. The first of these figures has 8 hexagonal and 6 square faces, making 14 plane surfaces in all; the second has 6 quadrilateral faces with curved sides, and the 8 hexagonal faces have their 6 corners and 3 long diagonals in one plane, but the surface between the diagonals curved. In this latter "minimal" tetrakaidecahedron the angles between adjacent faces are 120°. Kelvin solved his theoretical problem "on the division of space with minimum partitional area" by "homogeneously" dividing space into "equal and similar parts," "all sameways oriented." The tetrakaidecahedron was apparently known to the crystallographers before it was described by Kelvin, though the latter seems to have constructed it by himself as a result of his study of the soap film configuration of Plateau's cubic skeleton frame. Historically, then, Kelvin's tetrakaidecahedron was developed on the basis of liquid film studies, and to solve the problem of the division of space with minimum partitional area. Space-filling properties of the plane-faced tetrakaidecahedron have been analyzed by Matzke (1927).

In a series of investigations, Dewar (1916, 1917, 1918) studied soap films and bubbles, the latter singly and in relatively small groups. He recorded the occurrence of heptagons, hexagons, pentagons, and quadrilaterals, but gave no further data. Several of his figures suggest Kelvin's tetrakaidecahedra.

Simple and often ingenious experiments on soap bubbles were described by Boys (1890), while Lawrence (1929) took up in considerable detail some of the more theoretical aspects of soap film structure. Neither of these authors described the shape of massed bubbles. Detailed discussions of surface energy have been presented by Willows and Hatschek (1919), Rideal (1926) and Adam (1941). Harkins (1945) has recently dealt with this same topic, especially from the standpoint of solids.

The only serious attempt to study the three-dimensional shape of bubbles in foam was that of Desch (1919) who became interested in the problem through its bearing on the general topic of the form of the crystal grains in solidifying metals, as in marine propellors and crucible steel. Desch put soap solutions of various kinds into a glass trough with plane parallel sides and blew air through by means of a narrow pipette. He then counted as well as he could with the naked eye the number and kinds of faces on the "cells" thus formed. He also made a foam of warm gelatin, allowed this to solidify, and examined the "cells" at leisure. There was no accurate control of the size of the bubbles in these experiments, nor could the soap bubbles deep within a mass be studied in this way. According to his results, the "average number of faces to each cell" varied from 9 to 13, being higher in Plateau's solution than in gelatin. This is at variance with the data presented below. Desch was able to see that pentagonal faces were much more common than either hexagonal or quadrilateral faces, so that Kelvin's tetrakaidecahedron was not achieved under these conditions. He also noted that the cells often assumed the form of the pentagonal dodecahedron.

Furthermore, cells in contact with the surface glass of the containing vessel most commonly had 6 "sides." Desch concluded "that surface tension has an important share in determining the form of the crystal grains in a solidifying metal, and that such grains have a tendency to assume the shape of foam cells."

The three-dimensional shape of the alveoli of protoplasm has been discussed to some extent in biological literature, especially by Bütschli (1894) and by Seifriz. Bütschli thought that alveoli in a soap lather were mainly dodecahedral. In 1930 Seifriz stated that "protoplasmic alveoli, like the peas of Buffon, are rhombic dodecahedra." More recently, however (1936), he mentioned that their shape is "that of dodeca- or tetrakaidecahedrons."

Glaser (1938, 1941, 1945) and Glaser and Child (1937) have emphasized the importance of the tetrakaidecahedron and its aggregation series in the broader concepts of organic growth and have outlined applications at subcellular levels. Courant and Robbins (1941) discussed the significance of soap film studies in the solution of certain mathematical problems. Theoretical and practical aspects of foams were considered by Berkman and Egloff (1941).

Within the past three decades a number of digests of the whole topic of the three-dimensional forms of cells have been published. The first, by Thompson (1917), was rewritten in 1942. Some of Wilson's (1925) interpretations, especially those bearing on the cleavage of animal eggs, are particularly cogent. More recently, the whole general subject has been briefly presented by Lewis (1944, in Bremer-Weatherford) and by Matzke (1940).

It is obvious from the literature reviewed here that the problem of cell shape determination is one which concerns biologists in the analyses of form and development in plants and animals; it bears on the subject of protoplasmic structure; and in its ultimate solution it impinges upon such diverse topics as the theoretical division of space, the cooling of metals, and the action of surface forces in liquid films.

MATERIAL AND METHODS.—In the experiments described below a soap solution was used which was composed of triethanolamine oleate, glycerine, and distilled water. These are the substances previously utilized by Johnston (1935) in studies of torsional rigidity, and they give a soap solution similar to that of Dewar (1916) and to that which Trayer and March (1929) employed in their experimental study of the torsion of various types of beams used in aircraft construction. The proportions of these three materials were varied, and for the work here described, the following parts, by weight, were found most satisfactory: triethanolamine oleate 7.5 g., glycerine 34 g., distilled water 58.5 g. These substances were thoroughly shaken at intervals for several days and then allowed to stand for several weeks, or longer, before being used.

In the following experiments each bubble was made separately by means of a Yale tuberculin syringe, graduated to 1/100 cc. Most of the bubbles used had a volume of 1/10 cc. The plunger was therefore set at 1/10 cc., the very tip of the syringe was dipped into the soap solution, and the bubble blown by pressing down the plunger. After each bubble was made and placed into the dish used, excess solution was removed by wiping the tip of the syringe on cheesecloth. The bubbles were placed, one by one, into a cylindrical dish with an inside diameter of 6 cm. and an inside height of 6.5 cm. The inside of the dish was moistened with the soap solution before being used. A glass plate served as a cover.

When this work was first begun, the dish was filled to overflowing, the cover was placed down, breaking some of the bubbles, and sealed on in various ways. Due to temperature changes, expansion and contraction, this method did not prove satisfactory. Thereafter the dish was not filled to capacity; the cover was placed on top and held in position but was not sealed. Temperature changes then had no appreciable effect, for expansion would not bring the bubbles into contact with the top. Since the volume of the dish was 188 cc., it required nearly 1900 bubbles to fill it. During the course of the experiments described below, this dish was filled 16 times; each of the approximately 25,000 bubbles thus involved was made and placed into this dish separately. This technique was entirely different, therefore, from that used by Holman and by Desch, who simply inserted the end of a glass tube into a soap solution and blew, and then looked at the foam which resulted. Figure 1 is a photograph, looking down upon the dish, filled with bubbles of 1/10 cc.; figure 2 is a side view. As shown in figure 1, there were approximately 14 bubbles in one diameter at the surface.

In filling the dish it was necessary, from time to time, to revolve it, so that the solution would remain more or less uniformly distributed. If it were merely placed on a table and left, the walls of the bubbles would become thinner and thinner, and finally break. If the bubbles were to be kept overnight, the dish was placed on a clinostat and rotated on its long axis. This prevented breaking. If the dish were not rotated, the mass of bubbles might last, unbroken and intact, for a number of days—sometimes four or five—though individual ones would last much longer. On the clinostat they could be kept for extended periods. As Dewar and others have pointed out, freedom from contamination of the equipment and of the air is important in films that are to be kept for a long time. In the work here described the bubbles were always studied as soon after the dish was filled as possible—usually on the same day or on the following day. No attempt was made to place the bubbles in definite positions. They were put into the dish, one by one, uniformly, but without effort at spacing. They were

thus left free to adjust themselves, which they did. Precise spacing was not possible, because readjustments and slipping occurred. When the dish was turned and rotated to distribute the solution uniformly, further movement of the bubbles took place. However, a condition of relative equilibrium was finally achieved. While this was never absolute, the bubbles did remain fairly constant for some time. The same bubble could be singled out, in many cases, day after day, and the number and kinds of contacts usually did not change.

When the dish was filled and ready for use, the bubbles were examined with a Spencer binocular dissecting microscope using 1X paired objectives and paired 6X oculars. In some instances higher magnification was used. The advantage of using this type of microscope is that any bubble of the mass could be singled out and studied, regardless of its position in the dish. For example, if there were 1600 bubbles in the dish at one time, any one of these could be studied with precision, and the number of hexagonal, pentagonal, etc., faces recorded by merely focusing on that one bubble. Those above and below had little obscuring effect, provided the films were thin enough. It was not necessary to remove the cover of the dish. Use of the binocular dissecting microscope in this way, therefore, made possible the precise study of the bubbles in masses, and the compilation of the data presented below.

In the experiments here described, bubbles of equal volume were always used—the dish was always filled with the same sized bubbles. All the data on the peripheral (epidermal) bubbles were assembled when the dish was filled with bubbles having a volume, each, of 1/10 cc. Of the 600 central bubbles recorded below, data on 450 were collected when the dish was filled with bubbles of 1/10 cc.; data on 150 were compiled when the dish was filled with bubbles of 1/20 cc. Since the two sets of data on central bubbles, one set based on those having a volume of 1/10 cc. and the other on those with a volume of 1/20 cc., were practically identical, they have been combined in the tables below. Whether the volume was 1/10 or 1/20 of a cc. made no appreciable difference, provided that the dish was filled with bubbles all of the same volume.

There were obviously two kinds of peripheral bubbles, as is evident from figure 2—those in contact with the glass walls of the container and those free on the upper surface, or not in contact with the walls of the glass cylinder. After the first few trials, the dish was not filled as completely as is shown in figure 2, so that the cover could be placed upon it without breaking the bubbles on the surface.

It is evident from figure 2 that the bubbles at the base, which were in contact with the junction of the bottom and sides of the container, differed from those in contact just with the sides or in contact just with the bottom. In order to avoid this distorting effect, only bubbles at least four removed from the junction of the base and sides of the cylinder and at least four down from the top were used. Similarly, in studying the free peripheral bubbles on the upper surface, not in contact with the walls of the container, as shown in figure 1, only those were used which were at least four removed from the walls of the container. In figure 1, therefore, only those free peripheral bubbles were used which were toward the center.

Likewise, the data for the central bubbles were assembled from a "core" in the center of the whole mass of bubbles. No central bubble was tabulated unless it was at least four removed from the top of the mass, at least four removed from the base, and at least four removed from the side walls of the cylinder. The results of Marvin (1939a) on compression of lead shot indicate that only the surface layer is affected by flattening, but as a precaution, no central bubble was used unless it was separated from any surface by at least three others. Thus, although there were nearly 2000 bubbles in the mass shown in figure 2, a very much smaller number of central bubbles was available for compilation, under the conditions outlined above. Furthermore, if there was evidence of the breaking of the walls of bubbles—which sometimes happened—the whole mass was discarded. Because of all these limitations, it was necessary to fill the dish numerous times in the course of this work.

Figures 7 to 46 are drawings of individual bubbles, 7 to 26 of peripheral, and 27 to 46 of central bubbles. All were made with a Leitz camera lucida attached to the Spencer binocular dissecting microscope with 1X objective and 6X ocular. The drawing board was kept at constant height. Since the microscope had to be focused up and down for appreciable distances, especially in picking out the central bubbles in the dish, and since the drawing board was not correspondingly raised and lowered, the magnifications are not all precisely the same.

All these figures were made by plotting the corners of the faces and then connecting them with

TABLE 1. *Peripheral (epidermal) bubbles—number of faces.*

Not in contact with walls of cylinder			In contact with walls of cylinder		
No. of faces	No. of bubbles	Total faces	No. of faces	No. of bubbles	Total faces
7	2	14	7	0	0
8	13	104	8	0	0
9	17	153	9	3	27
10	42	420	10	46	460
11	48	528	11	106	1166
12	50	600	12	33	396
13	22	286	13	11	143
14	6	84	14	1	14
	200	2189		200	2206
Average $\frac{2189}{200} = 10.945$			Average $\frac{2206}{200} = 11.03$		

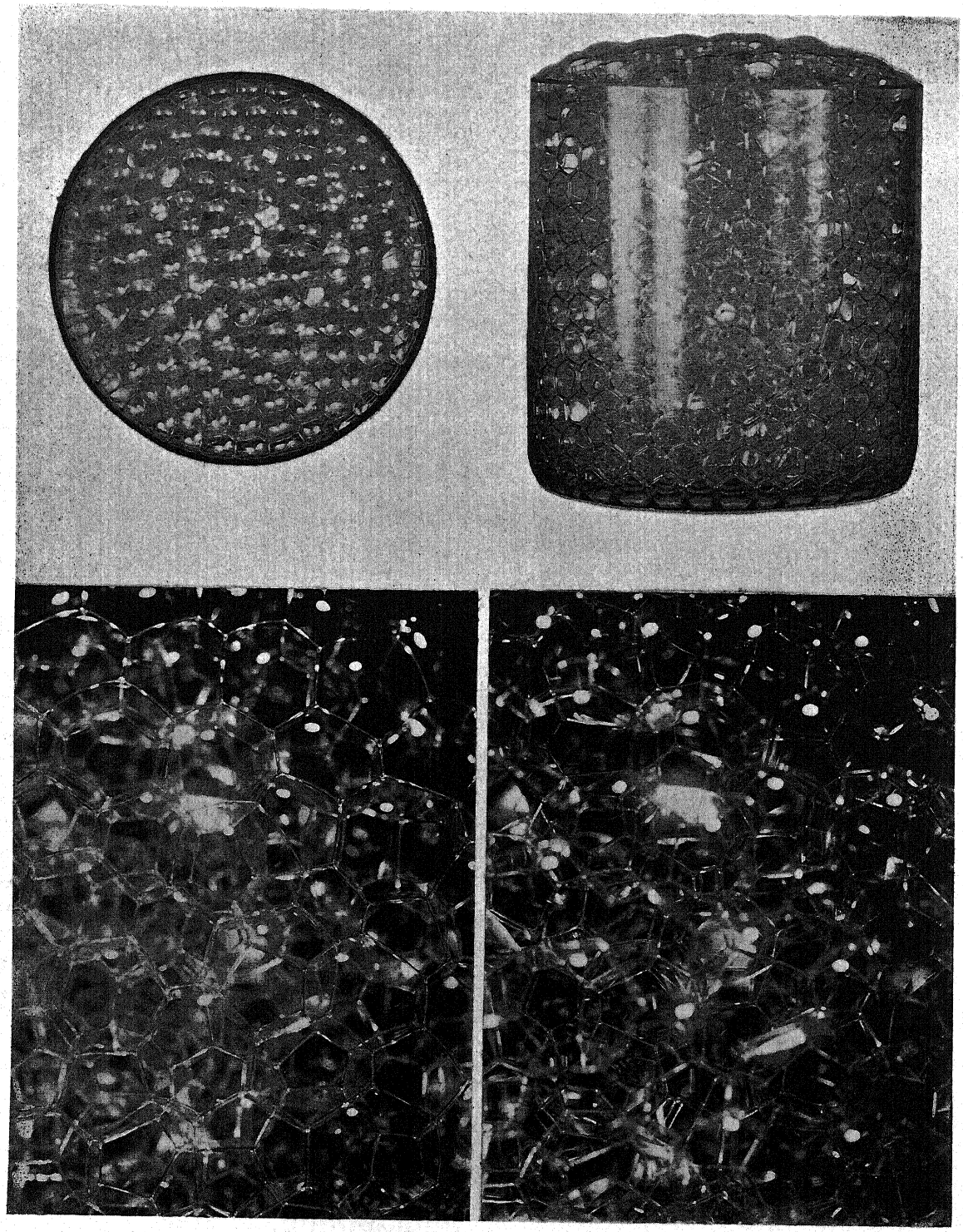


Fig. 1–4.—Fig. 1 (upper left). Dish filled with bubbles of 1/10 cc. volume. Top view. ×1.—Fig. 2 (upper right). Dish filled with about 2000 bubbles of 1/10 cc. volume. ×1.—Fig. 3 (lower left). Enlarged view of peripheral bubbles shown in upper portion of figure 1. Photographed with Zeiss photomicrographic camera with 30 mm. lens. ×3.4.—Fig. 4 (lower right). Central bubbles photographed with Zeiss photomicrographic camera with 30 mm. lens. ×3.4.

straight lines. The slight but beautiful delicate curvatures of the faces and edges, which may be seen in figures 1 to 4, therefore are not reproduced in these illustrations (fig. 7 to 46). To show these curvatures in line drawings of this type would be exceedingly difficult, and this simplification was

adopted merely as a matter of expediency. The number and kinds of contacts of each figure remain unaltered.

PERIPHERAL (EPIDERMAL) BUBBLES.—*Data.*—In all, 400 peripheral bubbles were studied and tabulated. Of these, 200 were free on the upper surface, limited in position as stated in the preceding paragraphs, and 200 were in contact with the walls of the cylinder as previously described. A somewhat enlarged view of some of the free peripheral bubbles is shown in figure 3, and the results are given in table 1. The average number of faces in each case was close to 11—10.945 for the free peripheral ones and 11.03 for those in contact with the walls of the cylinder. A closer inspection of these two sets of data, however, reveals that bubbles in contact with the walls of the cylinder were much more regular than those which were free on the upper surface. This would be expected, since some of those on the free surface were relatively deep, with just

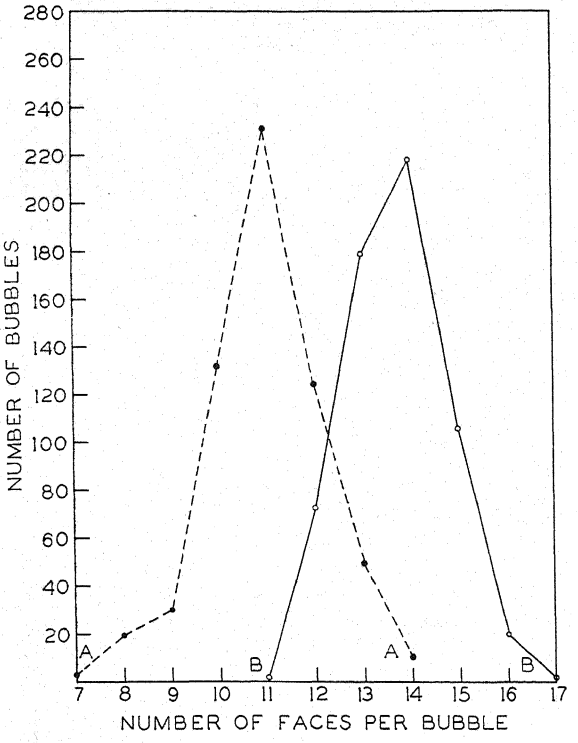


Fig. 5. Curves showing the number of faces per bubble and the frequency of the occurrence of bubbles with these varying numbers of faces in peripheral bubbles, AA, and in central bubbles, BB. Since 600 central bubbles were studied, and 400 peripheral ones, the numbers of the peripheral bubbles have been multiplied by 3/2 to make them comparable.

a small face exposed, and others were much less deeply set. This is also evident from figures 1, 2, and 3. Of the bubbles in contact with the walls of the cylinder, more than half (106) had 11 contacts, and of the total 200, there were 185 which had 10, 11, or 12 contacts. Those not in contact with the walls of the cylinder showed a greater range in the number of faces, and of the 200 studied, 140 had

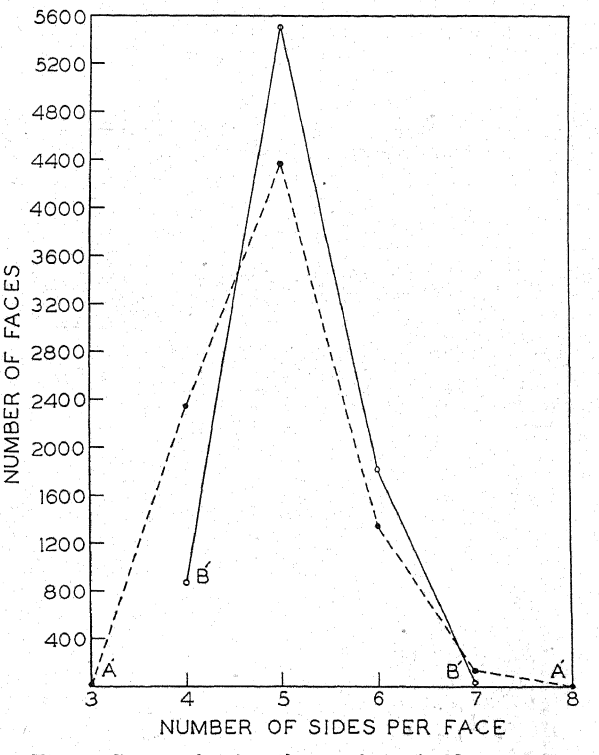


Fig. 6. Curves showing the number of sides per face (3—triangular, 4—quadrilateral, 5—pentagonal, etc.) and the frequency of occurrence of each kind of face in peripheral bubbles, A′A′, and in central bubbles, B′B′. Since there were 8221 faces in the central bubbles and 4395 in the peripheral ones, the numbers of the peripheral bubbles have been multiplied by 8221/4395 to make them comparable.

10, 11, or 12 faces. In table 2 these are both considered together, and the average for the 400 was 10.99—remarkably close to 11. For this total, the

TABLE 2. *Peripheral (epidermal) bubbles—number and kinds of faces.*

Totals of all peripheral bubbles				
No. of faces	No. of bubbles	Total faces	Kinds of faces	Total faces
7	2	14	Triangular	11
8	13	104	Quadrilateral	1252
9	20	180	Pentagonal	2338
10	88	880	Hexagonal	720
11	154	1694	Heptagonal	73
12	83	996	Octagonal	1
13	33	429		
14	7	98		4395
	400	4395		

Average $\frac{4395}{400} = 10.99$

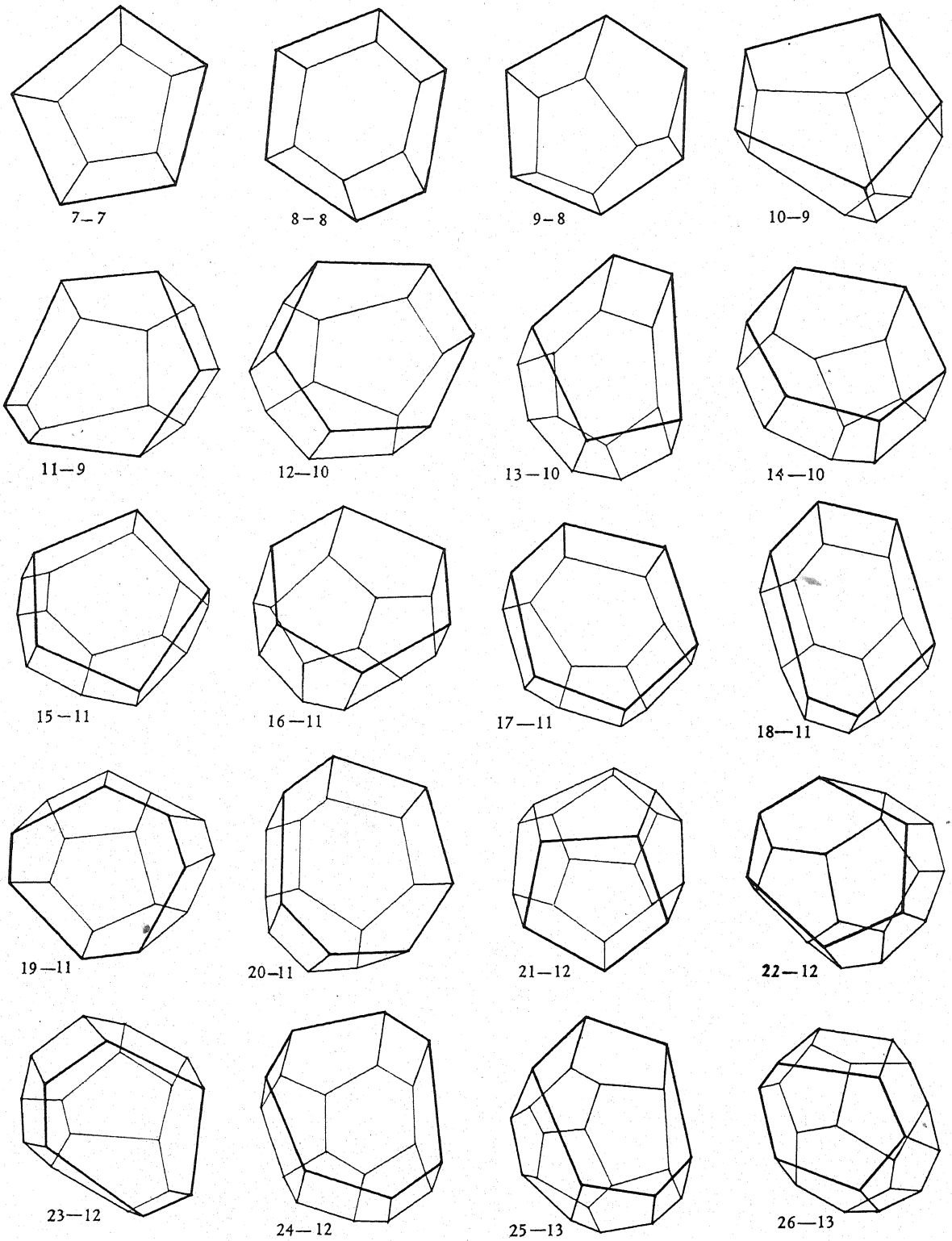


Fig. 7–26. Camera lucida drawings of peripheral bubbles. × c. 4.8. The second number under each figure indicates the total number of faces. Curvatures of faces and edges are not shown in the drawings. Triangular faces are designated as T; quadrilateral as Q, pentagonal as P, hexagonal as Hx, heptagonal as Hp. The combinations of faces in each are as follows:—Fig. 7. 7 faces, 5 Q, 2 P.—Fig. 8. 8 faces, 6 Q, 2 Hx.—Fig. 9. 8 faces, 1 T, 3 Q, 3 P, 1 Hx.—Fig. 10. 9 faces, 3 Q, 6 P.—Fig. 11. 9 faces, 4 Q, 4 P, 1 Hx.—Fig. 12. 10 faces, 3 Q, 6 P, 1 Hx.—Fig. 13. 10 faces, 3 Q, 6 P, 1 Hx.—Fig. 14. 10 faces, 4 Q, 4 P, 2 Hx.—Fig. 15. 11 faces, 2 Q, 8 P, 1 Hx.—Fig. 16. 11 faces, 2 Q, 8 P, 1 Hx.—

TABLE 3. *Combinations of faces found in 400 peripheral (epidermal) bubbles, and similar (matching) combinations in peripheral shot and cells.*

Number of sides per face						Total faces	Number of bubbles	Illus. in fig.	Similar (matching) combinations in		
3	4	5	6	7	8				106 periph. shot	550 undiff. cells	600 Elodea cells
.	5	2	..	..	..	7	2	7	0	1	73
..	4	4	..	..	..	8	2	..	2	2	6
..	6	0	2	..	..	8	5	8	0	0	228
1	3	3	1	..	..	8	6	9	0	0	0
..	3	6	..	..	..	9	2	10	2	3	13
..	4	4	1	..	..	9	11	11	4	2	15
..	5	2	2	..	..	9	4	..	1	8	3
..	7	0	0	2	..	9	1	..	0	0	59
1	4	2	1	1	..	9	2	..	1	0	0
..	2	8	..	..	..	10	3	..	0	6	0
..	3	6	1	..	..	10	29	12, 13	12	7	0
..	4	4	2	..	..	10	47	14	12	17	54
..	5	2	3	..	..	10	1	..	0	1	17
..	5	3	1	1	..	10	6	..	1	3	1
1	4	2	2	1	..	10	2	..	1	0	0
..	2	8	1	..	..	11	35	15, 16	2	5	0
..	3	6	2	..	..	11	67	17	8	14	1
..	4	4	3	..	..	11	22	18	7	16	0
..	5	2	4	..	..	11	1	..	0	4	0
1	3	3	4	..	..	11	1	..	2	0	0
..	4	5	1	1	..	11	22	19	1	2	11
..	5	3	2	1	..	11	6	20	1	1	1
..	..	12	..	..	..	12	11	21	0	0	0
..	2	8	2	..	..	12	19	22	0	9	0
..	3	6	3	..	..	12	22	23	2	14	0
..	4	4	4	..	..	12	9	..	1	16	5
..	3	7	1	1	..	12	13	24	2	4	0
..	4	5	2	1	..	12	3	..	1	7	0
..	4	6	0	2	..	12	1	..	1	2	0
..	5	3	3	1	..	12	2	..	1	5	1
..	5	4	1	2	..	12	2	..	0	4	1
..	6	1	4	1	..	12	1	..	0	0	0
..	1	10	2	..	..	13	17	25	0	1	0
..	2	8	3	..	..	13	5	26	0	6	0
..	3	6	4	..	..	13	3	..	0	20	0
..	2	9	1	1	..	13	2	..	0	2	0
..	3	7	2	1	..	13	3	..	0	4	0
..	4	5	3	1	..	13	2	..	0	5	0
..	4	6	2	0	1	13	1	..	0	0	0
..	..	12	2	..	..	14	1	..	0	0	0
..	1	10	3	..	..	14	2	..	1	1	0
..	2	8	4	..	..	14	3	..	0	5	0
..	2	9	2	1	..	14	1	..	1	1	0
							400		67	198	489

number of faces forms a relatively symmetrical curve, as shown in figure 5,AA.

Table 2 and figure 6,A′A′ show the number of triangular, quadrilateral, pentagonal, etc., faces which occurred in these 400 bubbles. It is immediately obvious that more than half of the faces were pentagonal and that the very great majority were either quadrilateral, pentagonal, or hexagonal. This

Fig. 17. 11 faces, 3 Q, 6 P, 2 Hx.—Fig. 18. 11 faces, 4 Q, 4 P, 3 Hx.—Fig. 19. 11 faces, 4 Q, 5 P, 1 Hx, 1Hp.—Fig. 20. 11 faces, 5 Q, 3 P, 2 Hx, 1 Hp.—Fig. 21. 12 faces, 12 P.—Fig. 22. 12 faces, 2 Q, 8 P, 2 Hx.—Fig. 23. 12 faces, 3 Q, 6 P, 3 Hx.—Fig. 24. 12 faces, 3 Q, 7 P, 1 Hx, 1 Hp.—Fig. 25. 13 faces, 1 Q, 10 P, 2 Hx.—Fig. 26. 13 faces, 2 Q, 8 P, 3 Hx.

is much greater uniformity than Marvin (1939a) found in the peripheral layer of compressed lead shot.

Record was kept, in each of the 400 peripheral bubbles studied, of the number of contacts, the kinds of contacts, and for each one the combination of faces. These combinations are given in table 3. There were 43 of these combinations in all, 20 of which are illustrated in figures 7 to 26. Although bubbles with 11 faces were far more numerous than any other kind (table 2), only 7 different combinations of faces were found among the 154 studied, while 10 different combinations occurred in the 83 with 12 facets. The relative uniformity among those with 11 faces can be accounted for, in part at least, by the fact that almost half of those (67) had a single combination of faces—3 quadrilateral, 6 pentagonal, and 2 hexagonal (fig. 17). This foam cell, with a hexagonal face above, with 6 lateral contacts, and with 4 basal ones, constituted 17 per cent of the total number studied. While this is hardly frequent enough to constitute "the type," it is nevertheless a form that was commonly achieved. The next most common combination, a 10-faceted cell with 4 quadrilateral, 4 pentagonal, and 2 hexagonal faces, occurred 47 times and is illustrated in figure 14. Like the one illustrated in figure 17, it has a hexagonal face above, and 6 lateral contacts, but 3 instead of 4 basal ones. The third most abundant form is illustrated in figures 16 and 15. Each of these has 2 quadrilateral, 8 pentagonal, and 1 hexagonal face—a total of 11. In figure 16 there is a hexagonal face above, and there are 6 lateral contacts and 4 basal ones; this is the same arrangement as in the commonest combination, illustrated in figure 17, though the latter has 2 hexagonal faces. The foam cells shown in figures 15 and 16, though identical in number and kinds of faces, differ in that the one shown in figure 16 has a hexagonal face above, while the one in figure 15 has a pentagonal face on its free surface. Figures 12 and 13 again illustrate foam cells with the same combination of faces—3 quadrilateral, 6 pentagonal, and 1 hexagonal—10 faces in all; but in figure 12 there is a hexagonal face above, while in figure 13 a pentagonal face is on top. The four different combinations of faces illustrated in the six figures 12, 13, 14, 15, 16, 17 occurred altogether 178 times among the total of 400 bubbles studied (table 3). Other commonly occurring combinations are shown in figures 11, 18, 19, 22, 23, 24, and 25.

The foam cell shown in figure 7 is really a modified pentagonal prism, while that shown in figure 8 is a modified hexagonal prism. In figure 9 can be seen one of the small triangular faces occasionally found.

In the literature there is considerable discussion of the pentagonal dodecahedron, which has 12 faces, all pentagons. It was found 11 times among these peripheral bubbles (table 3, fig. 21). This, as well as the type shown in figure 26, occurred especially among peripheral foam cells that were sunk fairly deep down, but with one relatively small face at the surface. The pentagonal dodecahedra were not necessarily regular, and the walls of these as well as of the other bubbles frequently showed curvatures.

Discussion.—It is evident from the data presented in tables 1, 2, and 3 that the peripheral bubbles of a mass had an average of 11 faces (10.99 for 400 bubbles); although the range was from 7 to 14 faces, 38.5 per cent of the 400 studied had 11. Pentagonal faces were more common than all other types combined, and while the range was from triangular to octagonal, 98 per cent of the facets were either quadrilateral, pentagonal, or hexagonal.

Among the 154 11-faceted foam cells, one kind occurred 67 times (fig. 17). This was the most frequent combination, but it seems hardly justifiable under the conditions of the experiments here outlined to call this "the type" of peripheral foam cell, since it was one of 43 different combinations. No one form among those found rightly can be called "the type," although the one shown in figure 17 comes closer to justifying such a designation than any other.

Marvin (1939a) compressed lead shot in a steel cylinder, to eliminate all interstices, and then picked apart the lead pellets and examined them. He found that 106 peripheral shot had an average of 10.75 contacts. This average is fairly close to the 10.99 obtained for peripheral foam cells. His range was from 8 to 14 contacts, again paralleling the data on bubbles rather closely. Similarly, he found that the kinds of faces varied from triangular to octagonal, the great majority being quadrilateral, pentagonal, and hexagonal, with pentagons most abundant.

However, a closer inspection of Marvin's data shows that although compressed lead shot and bubbles in foam had certain features in common, there were also definite and important differences. While pentagonal faces were the most frequent, they were not more abundant than all others combined, as in the case of the peripheral bubbles. But the most striking difference was in the combinations of faces found in the peripheral bubbles (table 3) and in the peripheral shot.[2] A total of 400 peripheral bubbles had 43 different combinations of faces; a total of 106 peripheral shot had 52 different combinations of faces. There was, then, much less uniformity in the shot than in the bubbles. Similarly, the most abundant combination occurred 67 times in the bubbles (fig. 17). This was found in the 106 shot 8 times. The commonest combinations in the shot were found 12 times, and they are the ones illustrated by figures 12 and 14 in the bubbles. Of the 52 combinations in the shot, 33 occurred only once, while only 9 of the 43 peripheral bubble combinations were

[2] Professor James W. Marvin has kindly permitted an analysis to be made of his complete data on compressed lead shot, which are published in summary form (Marvin, 1939a). Some of the comparisons made are based on his very lengthy unpublished tables.

found a single time. In table 3 are listed the peripheral shot which had the same combinations as the peripheral bubbles. A total of 67 of the 106 shot fell into the same categories, 39 did not. While it is true that some of the commoner bubble combinations were also fairly well represented in the shot, other common patterns occurred rarely or not at all in the shot. The combination that was by far the most abundant in the bubbles was the third most frequent in the shot. It is evident from these comparisons that there was much less constancy in the combinations found in the shot than in those in the foam cells, and that in the shot the combinations extended over a much greater range. This more pronounced constancy in the foam cells can be ascribed, in part at least, to the greater effectiveness of the surface forces in soap films. The geometrical requirements are to some extent similar in both the shot and the bubbles. Although the geometrical limitations cannot be circumvented, other factors which are also present are responsible for differences which appear and which are important.

Marvin reported 12 tetrahedral angles in the 106 peripheral shot, and, as demonstrated by Plateau in 1873 and later by Kelvin, these do not occur in soap films; none were found in the present work.

Thus far no statistical data are available on epidermal cells, although investigations are under way; consequently, comparisons between cells and peripheral bubbles cannot be made in complete fashion at present. However, certain commonly recurring patterns can be pointed out.

The cells available on which complete statistical data have been published are as follows: *Eupatorium purpureum* 100 pith cells (Marvin, 1939b), *Eupatorium perfoliatum* 100 pith cells (Marvin, 1944), *Angiopteris evecta* 100 petiole parenchyma cells (Higinbotham, 1942), *Citrus grandis* 100 carpel vesicle cells (Dodd, 1944), *Ailanthus glandulosa* 150 pith cells (Hulbary, 1944). Limited comparisons are also possible with 600 differentiated cortical cells of *Anacharis densa* (*Elodea*) (Hulbary, 1944). There are thus 550 essentially undifferentiated cells and 600 differentiated *Elodea* cortical cells available, but no epidermal cells.

As shown in table 3, the 400 peripheral bubbles occurred in 43 different combinations of faces. The 550 undifferentiated cells were found in 203 different combinations of faces. Of these undifferentiated cells, 198 had the same combinations of faces as peripheral bubbles (table 3), which were found in 43 combinations. Of these same 550 undifferentiated cells, 141 had combinations of faces similar to (matching) the 52 combinations that Marvin recorded for 106 peripheral shot. In the differentiated cortical cells of *Elodea* studied by Hulbary the results were similar. Of these 600 *Elodea* cells, which occurred in 31 different combinations of faces, 489 matched the 43 bubble combinations; 116 of these same 600 cells matched the 52 peripheral shot combinations. Although the number of combinations in the peripheral shot was greater than in the bubbles (52 compared with 43), still a very much greater number of cells matched the bubbles. This strongly suggests that surface forces, which are of far greater importance in effecting the form of the foam cells than of the compressed lead shot, also play a rôle in determining the shapes of the cells.

The combination most frequently occurring in the peripheral bubbles—3-6-2 (fig. 17 and table 4)—has been reported 14 times in the undifferentiated cells listed above and once in the *Elodea* cortex. It was one of the commonest combinations in the cells studied, being exceeded only by four others among 203 thus far found. This takes on added significance in view of the fact that the cell data are not from epidermal cells. The second most abundant combination in the foam—4-4-2 (fig. 14)—occurred 17 times in the undifferentiated cells thus far described and was the fourth commonest in the 600 *Elodea* cells, having been found there 54 times. It was also one of the two commonest combinations in Marvin's (1939a) peripheral shot. The third most common form of bubble—2-8-1 (fig. 15, 16)—was found 35 times, 5 times in the undifferentiated cells and only twice in the peripheral shot. The pattern

TABLE 4. *Most commonly occurring combinations of faces in peripheral (epidermal) bubbles, and similar (matching) combinations in peripheral shot and cells.*

Number of sides per face				Total faces	Number of bubbles of 400	Illus. in fig.	Similar (matching) combinations in		
4	5	6	7				106 periph. shot	550 undiff. cells	600 Elodea cells
3	6	2	..	11	67	17	8	14	1
4	4	2	..	10	47	14	12	17	54
2	8	1	..	11	35	15, 16	2	5	0
3	6	1	..	10	29	12, 13	12	7	0
4	4	3	..	11	22	18	7	16	0
4	5	1	1	11	22	19	1	2	11
3	6	3	..	12	22	23	2	14	0
2	8	2	..	12	19	22	0	9	0

illustrated in figures 12 and 13 (3-6-1), which occurred 29 times in the foam, was one of the two most abundant in the peripheral shot and was found 7 times in undifferentiated cells. A further inspection of table 4 indicates that the commonest combinations occurring in the peripheral bubbles were also well represented in the undifferentiated cells and much less commonly in the peripheral shot. This is especially obvious in the last three combinations, which occurred 63 times altogether in the bubbles, were matched by 25 undifferentiated and 11 cortical cells, and were found in only three instances in the peripheral shot. This is in spite of the fact that the cells here considered were not epidermal, no data on such as yet being available.

It is also significant that the combinations that Hulbary (1944) found to be most common in the differentiated cortical cells of *Elodea* also occurred in the peripheral bubbles. Of his 600 *Elodea* cells, 489 were matched by 189 peripheral bubbles (table 3). All his most numerous ones were matched by bubbles—5-2 (fig. 7), 6-0-2 (fig. 8), 7-0-0-2, and 4-4-2 (fig. 14). The first three of these were unmatched by peripheral shot, and the fourth was found 12 times in the peripheral shot. This suggests strongly that even in those rather specialized *Elodea* cells, surface forces must be considered as one of the factors responsible for their three-dimensional cell shape determination.

In various publications, Lewis (1928b, 1930, 1931, 1937) has discussed the epidermis of *Cucumis* and *Tradescantia* and has pointed out how "a simple epithelium resting on a layer of cells of the same diameter" has 11-faceted cells. Such cells may have 4 basal and 6 lateral contacts and the free outer face. More recently, however, Lewis (1943b) has pointed out that if the plant is regarded as a cylindrical shaft terminating above and below in a hemispherical tip, and if this shaft is "covered with an epidermal mosaic presenting only 3-rayed vertices, and consequently consisting of cells with an average of precisely six sides, the two extremities (if also showing only 3-rayed vertices) must be covered with cells having altogether twelve fewer sides than if they were all hexagons." In his latest publication Lewis (1944) has shown how compensating variations may occur between epidermis and underlying parenchyma. In the data on peripheral bubbles presented above, however, the complete cylindrical shaft was not used; the bubbles at or near the junction of base and sides or of top and sides were not studied; and the average for the 400 peripheral bubbles tabulated was 10.99. This is as close to 11 as can be expected under experimental conditions.

A comparison of all these data on peripheral bubbles, peripheral compressed shot, and on the cells thus far studied and tabulated—epidermal cells not being available as yet—leads inevitably to certain conclusions. Geometrical considerations are present in all three of these systems, and they cannot be circumvented. However, the bubbles show much greater uniformity than the shot, due presumably to the importance of surface forces, in addition to the geometrical necessities. Furthermore, the available cell data show conclusively that the cells are more similar to the bubbles than to the shot. This is evident especially when the combinations of faces on peripheral bubbles, peripheral shot, and in cells are compared. It may be deduced that surface forces are effective in the cells when their shape is determined. Although other factors also may be responsible in part for this greater similarity of cells to bubbles, no other seems as obvious.

CENTRAL BUBBLES.—*Data.*—As pointed out in the paragraphs on material and methods above, the bubbles were made either 1/10 or 1/20 of a cc. in volume, but the dish was always filled with bubbles of one size at one time. The data were practically the same whether the volume was 1/10 cc. or 1/20 cc., and the results are therefore considered together in the tables. By central bubbles are meant those that were separated from the top, bottom, and sides of the cylinder by at least three other bubbles.

TABLE 5. *Central bubbles—number and kinds of faces.*

No. of faces	No. of bubbles	Total faces	Kinds of faces	Total faces
11	2	22	Quadrilateral	866
12	73	876	Pentagonal	5503
13	179	2327	Hexagonal	1817
14	218	3052	Heptagonal	35
15	106	1590		
16	20	320		8221
17	2	34		
	600	8221		

Average $\frac{8221}{600} = 13.70$

From table 5 it is obvious that the average number of contacts of the 600 central bubbles was 13.70. Of these, 300, of 1/10 cc. by volume, were tabulated several years ago, and their average number of contacts was 13.69. Recently 150 additional ones with a volume of 1/10 cc. were studied, and they averaged 13.69 faces; then 150 more, of 1/20 cc., were tabulated, and their average number of contacts was 13.74. The average for the 600 was 13.70. This is close to 14, but since the figures are in such strong agreement, the deviation from 14 is apparently not without meaning.

The range in number of contacts was from 11 through 17, with the greatest frequency at 14 (table 5 and fig. 5,BB). More than a third of the 600 foam cells had 14 faces, and more than 5/6 (503 altogether) had 13, 14, or 15 contacts. In comparison with results in other kinds of systems (compressed shot and cells), this range was narrow.

Of the total of 8221 faces, more than 2/3 (5503)

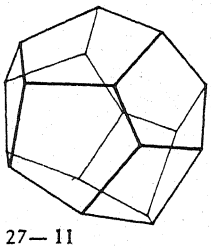
TABLE 6. *Combinations of faces found in 600 central bubbles, and similar (matching) combinations in central shot and undifferentiated cells.*

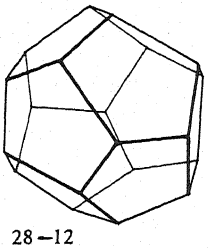
Number of sides per face				Total faces	Number of bubbles	Illus. in fig.	Similar (matching) combinations in	
4	5	6	7				624 central shot	550 undiff. cells
3	6	2	..	11	2	27	0	14
..	12	..	..	12	50	28	3	0
2	8	2	..	12	15	..	10	9
3	6	3	..	12	7	..	9	14
4	4	4	..	12	1	29	3	16
1	10	2	..	13	118	30	5	1
2	8	3	..	13	19	31	12	6
3	6	4	..	13	36	32	16	20
4	4	5	..	13	4	..	7	10
5	2	6	..	13	1	..	4	5
3	7	2	1	13	1	33	2	4
..	12	2	..	14	39	34	2	0
1	10	3	..	14	73	35	3	1
2	8	4	..	14	64	36	17	5
3	6	5	..	14	17	37	16	5
4	4	6	..	14	12	38	13	9
2	9	2	1	14	4	..	2	1
3	7	3	1	14	8	39	8	10
4	5	4	1	14	1	..	15	13
..	12	3	..	15	21	40	1	0
1	10	4	..	15	35	41	3	1
2	8	5	..	15	24	42	4	2
3	6	6	..	15	7	..	5	4
4	4	7	..	15	2	43	1	8
1	11	2	1	15	2	..	1	0
2	9	3	1	15	6	44	1	3
3	7	4	1	15	5	..	7	6
4	5	5	1	15	2	..	4	9
5	3	6	1	15	2	..	3	4
..	12	4	..	16	10	45	0	0
1	10	5	..	16	4	..	0	0
2	8	6	..	16	1	..	2	5
3	6	7	..	16	1	..	5	1
2	9	4	1	16	3	46	1	1
3	7	5	1	16	1	..	3	4
2	8	7	..	17	2	..	0	1
					600		188	192

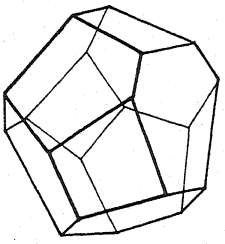
were pentagonal, and 99.6 per cent of all the faces were either quadrilateral, pentagonal, or hexagonal (table 5 and fig. 6,B'B'). Here again there was a relatively narrow range, with a great preponderance of pentagonal facets.

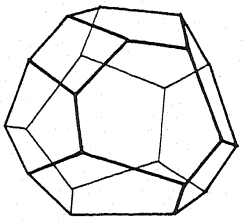
The total number of combinations of faces found in the 600 central bubbles is given in table 6, and the commonest ones are illustrated in figures 27 to 46. It is evident from table 6 that 36 different combinations occurred in these 600 central bubbles. This contrasts with 43 combinations for 400 peripheral bubbles, with 303 combinations for Marvin's (1939a) 624 central shot, and with 203 combinations for 550 undifferentiated cells. The central bubbles were obviously much more constant in their combinations of faces than either the shot, which were of uniform volume, or the cells, which varied in volume considerably.

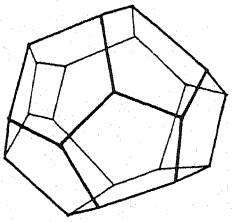
An inspection of table 6 reveals that, although bubbles with 14 facets were more numerous than any others, the most abundant combination was a 13-faceted foam cell with 1 quadrilateral, 10 pentagonal, and 2 hexagonal faces (fig. 30). This occurred 118 times among the 600 bubbles studied and was thus much more common than any other (table 7). It had a great preponderance of pentagonal faces. From table 6 it can be seen that bubbles with large numbers of pentagonal faces were normally the common ones. This contrasts strongly with Kelvin's tetrakaidecahedra, in which the faces

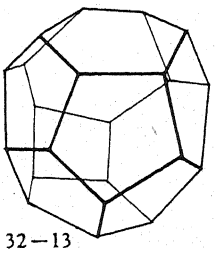

27—11

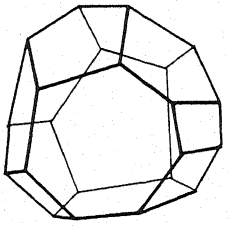

28—12

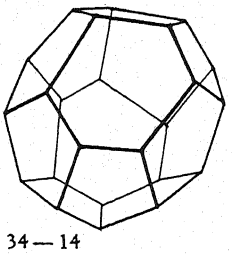

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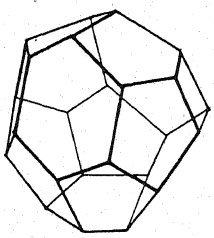

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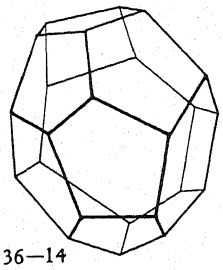

31—13

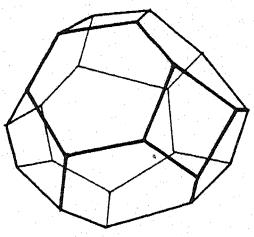

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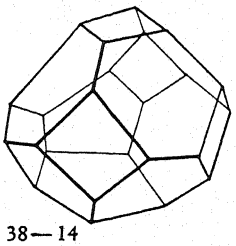

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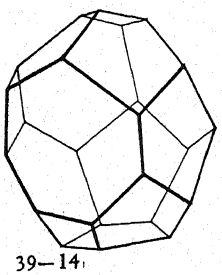

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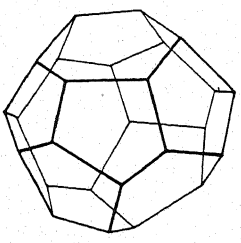

35—14

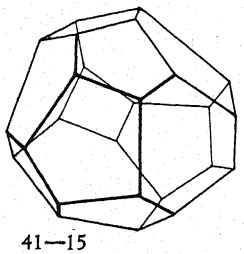

36—14

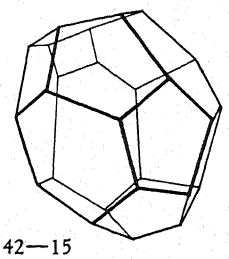

37—14

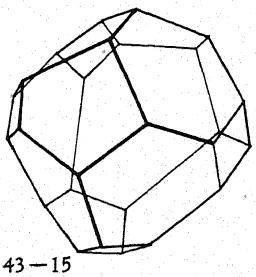

38—14

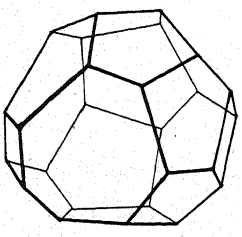

39—14

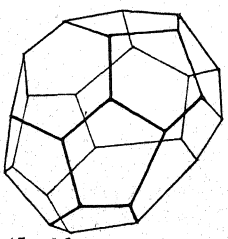

40—15


41—15


42—15


43—15


44—15


45—16

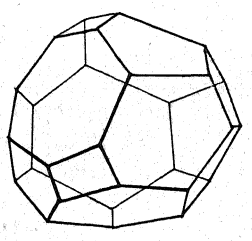

46—16

Fig. 27–46. Camera lucida drawings of central bubbles. × c. 4.4. The second number under each figure indicates the total number of faces. Curvatures of faces and edges are not shown in the drawings. Quadrilateral faces are designated as Q, pentagonal as P, hexagonal as Hx, heptagonal as Hp. The combinations of faces in each are as follows:—Fig. 27. 11 faces, 3 Q, 6 P, 2 Hx.—Fig. 28. 12 faces, 12 P.—Fig. 29. 12 faces, 4 Q, 4 P, 4 Hx.—Fig. 30. 13 faces, 1 Q, 10 P, 2 Hx.—Fig. 31. 13 faces, 2 Q, 8 P, 3 Hx.—Fig. 32. 13 faces, 3 Q, 6 P, 4 Hx.—Fig. 33. 13 faces, 3 Q, 7 P, 2 Hx, 1 Hp.—Fig. 34. 14 faces, 12 P, 2 Hx.—Fig. 35. 14 faces, 1 Q, 10 P, 3 Hx.—Fig. 36. 14 faces, 2 Q, 8 P.

TABLE 7. *Most commonly occurring combinations of faces in central bubbles, and similar (matching) combinations in central shot and undifferentiated cells.*

Number of sides per face			Total faces	Number of bubbles of 600	Illus. in fig.	Similar (matching) combinations in 624 central shot	Similar (matching) combinations in 550 undiff. cells
4	5	6					
1	10	2	13	118	30	5	1
1	10	3	14	73	35	3	1
2	8	4	14	64	36	17	5
..	12	..	12	50	28	3	0
..	12	2	14	39	34	2	0
3	6	4	13	36	32	16	20
1	10	4	15	35	41	3	1
2	8	5	15	24	42	4	2
..	12	3	15	21	40	1	0
2	8	3	13	19	31	12	6

are all quadrilaterals and hexagons, with no pentagons at all. The second and third most common combinations, 1-10-3 (fig. 35) and 2-8-4 (fig. 36), were both bubbles with 14 faces. These again showed a high proportion of pentagonal faces, and together they accounted for 137 of the total of 600 bubbles. The fourth most common type was the pentagonal dodecahedron, with 12 faces, all pentagons, which occurred 50 times (fig. 28). The constant recurrence of this form in all the work on bubbles and foams commands attention. The faces and edges were frequently curved in the soap films, so that this polyhedron was not the completely regular pentagonal dodecahedron, but it was easily recognizable nevertheless. Although there were 36 different combinations among the 600 foam cells, these first four (table 7) accounted for 305, or more than half. This tends further to emphasize the relative constancy in these soap film patterns. The fifth combination, in order of frequency, was 0-12-2 (fig. 34), another 14-faced cell, which was found 39 times. Among the first five combinations, therefore, three were 14-faced cells. Sixth in order of frequency was another 13-faceted foam cell, 3-6-4 (fig. 32), and following this were three combinations with 15 faces, 1-10-4 (fig. 41), 2-8-5 (fig. 42), and 0-12-3 (fig. 40). The tenth most common combination, 2-8-3 (fig. 31), was found 19 times. These first 10 patterns (table 7) accounted for 479 of the total of 600 bubbles, or 80 per cent. And of these, 176 had 14 faces, 173 had 13 faces, 80 had 15 faces, and 50 had 12 faces. Furthermore, one of these combinations had just one type of face—all pentagons; two had two kinds of faces—pentagons and hexagons; and the other 7 had three kinds of faces—quadrilaterals, pentagons, and hexagons. Not a single heptagonal face occurred among these 10 commonest combinations.

The bubble with 16 faces which was found most frequently had 12 pentagonal and 4 hexagonal faces (fig. 45). It occurred 10 times among 600. Figure 46 shows a 16-faceted foam cell, found three times, with one of the less common heptagonal faces. Other heptagonal faces are shown in figures 33, 39, and 44. The only combination with 11 faces—3-6-2—was found twice and is shown in figure 27. In figure 29 a 12-faceted foam cell is illustrated having the formula 4-4-4, thus achieving one kind of symmetry, although it was found only once. Fourteen-faced bubbles of common occurrence, not previously discussed, are shown in figures 37 and 38. The bubble shown in figure 43 had relatively few pentagonal faces—4-4-7—although it was exceeded in this respect by several other combinations, which thus came closer to Kelvin's tetrakaidecahedron.

Discussion.—There can be little question that the penetrating essays of Kelvin (1887, 1894) "On the Division of Space with Minimum Partitional Area" and "On Homogeneous Division of Space" have been of vital importance in clarifying the concepts of three-dimensional cell shapes, for they have afforded a scientific basis for considering a 14-faced polyhedron as a fundamental type, rather than the dodecahedron which was almost universally accepted before, and for a long time afterward. It may also be recalled that Kelvin arrived at his tetrakaidecahedra as the result of a consideration of soap films and surface forces. While the theoretical validity of Kelvin's figure for the division of space, "homogeneously," with "minimum partitional area," into "equal and similar parts" which are "all sameways oriented" may not be questioned, it is possible to read into his conclusions certain broad generalizations which do not necessarily apply. In speaking of Kelvin's "minimal" tetrakaidecahedron, Thompson (1942) states "We may

4 Hx.—Fig. 37. 14 faces, 3 Q, 6 P, 5 Hx.—Fig. 38. 14 faces, 4 Q, 4 P, 6 Hx.—Fig. 39. 14 faces, 3 Q, 7 P, 3 Hx, 1Hp.. —Fig. 40. 15 faces, 12 P, 3 Hx.—Fig. 41. 15 faces, 1 Q, 10 P, 4 Hx.—Fig. 42. 15 faces, 2 Q, 8 P, 5 Hx.—Fig. 43. 15 faces, 4 Q, 4 P, 7 Hx.—Fig. 44. 15 faces, 2 Q, 9 P, 3 Hx, 1 Hp.—Fig. 45. 16 faces, 12 P, 4 Hx.—Fig. 46, 16 faces, 2 Q, 9 P, 4Hx, 1 Hp.

take it as certain that in a homogeneous system of fluid films like the interior of a froth of soap-bubbles, where the films are perfectly free to glide or turn over one another and are of approximately coequal size, the mass is actually divided into cells of this remarkable conformation." It is quite possible that under "ideal" conditions involving *perfect spacing* of equal and similar foam cells, Kelvin's figure might be realized. On the other hand, there is no doubt that if 2000 soap bubbles, all of equal volume, are placed into a cylindrical dish, one by one, as described above, and are allowed to slip and adjust themselves, Kelvin's figure, with 8 hexagonal and 6 quadrilateral faces, is not realized. Although the average number of contacts for 600 central bubbles was fairly close to 14 (13.70), not a single one of these had 8 hexagonal and 6 quadrilateral faces. And of the 400 peripheral bubbles recorded, the same was true. A study of tables 3 and 6 shows immediately that the combinations of faces that came closest to Kelvin's figure were not common; for instance, the combinations 5-2-6 and 5-3-6-1 were found only once and twice, respectively. It is obvious that the bubbles with the greatest number of pentagonal faces, and in this respect least like Kelvin's figures, were the most numerous by far. There can be no question that under the conditions portrayed above, no one "type" is achieved, although certain patterns or combinations of faces recur much more commonly than others; and they are not the ones that Kelvin described.

Plateau's conclusions seem perfectly valid. There were never more than three films meeting in an edge, and the films certainly tended to meet at angles of 120°. Similarly, the edges, through curvatures (seen in figures 1 to 4, but not shown in figures 7 to 46) tended to be arranged at angles of 109°28′16″, as Plateau pointed out. And "tetrahedral angles" did not occur. That the conditions postulated by Plateau can be approximated, if not achieved, by foam cells not as regular as Kelvin described, seems obvious. The measurements of Marvin (1939b) show that irregular cells may approach, and in individual instances actually surpass, in economy of surface, the orthic tetrakaidecahedron of Kelvin. Whether or not the somewhat irregular partitioning of space into such dissimilar cells can ever achieve the economy of surface that Kelvin's tetrakaidecahedra attain is a mathematical problem. However, if Plateau's conditions are approximated or achieved in somewhat irregular cells, and if a relatively great economy of surface with reference to volume is attained in these foam cells, then, even if the minimal tetrakaidecahedron of Kelvin does have a slightly greater economy of surface, its realization would not be expected unless the surface forces were relatively quite powerful. Kelvin's presentation is philosophical. Even in soap films, as here studied, other forces such as adhesion and gravity are still present. However, a mass of bubbles of equal volume presents a much greater uniformity of pattern than a mass of compressed lead shot of uniform volume.

It is certainly possible to conceive of space being divided into somewhat irregular units (cells) of equal volume, with stable angles between surfaces and edges (as outlined by Plateau) and showing economy of surface to volume (perhaps not maximum economy). Such a division of space was present in the foam cells here described. With *perfect spacing,* Kelvin's tetrakaidecahedra might be achieved.

From table 5 it is immediately obvious that pentagonal faces were much more numerous in the central bubbles than all others added together. A further study of table 6 shows that whether the polyhedra[3] were 12-faceted, 13-faceted, 14-faceted, 15-faceted, or 16-faceted, the ones with the greatest number of pentagonal faces were the common ones. In the 12-hedra, the pentagonal dodecahedron occurred more than twice as often as all the others added together; among the 13-hedra, the combination 1-10-2 occurred 118 times—almost twice as often as the sum of all the other 13-hedra found; among the 14-hedra the ones most often found had numerous pentagonal faces. It is evident from these data that polyhedra with pentagonal faces predominated. In spite of this preponderance of pentagonal faces, certain combinations which would be expected failed to occur. As may be seen from tables 3 and 6, the combinations 1-10-0, 1-10-1, and 0-12-1 were not found.

The pentagonal dodecahedron was recorded 50 times among the 600 bubbles studied. The regular pentagonal dodecahedron—and the ones found were not all perfectly regular—comes close to satisfying Plateau's conditions. The angles between faces are 116°33′54″—close to the required angle of 120°; and the edges form angles of 108°—again approaching closely to the required angle of 109° 28′16″. Furthermore, there are always three edges meeting in a point, forming trihedral angles. This figure, therefore, requires only little modification to satisfy the equilibrium conditions. However, since faces do not meet at 120° angles, space cannot be filled with the regular figure, and, therefore, it was discarded by Kelvin. In the somewhat irregular partitioning of space found under the conditions described, pentagonal dodecahedra occurred frequently.

In addition to the conditions of Plateau involving stability of angles as outlined above, one other characteristic of the regular pentagonal dodecahedron merits consideration. In any system of partitioning of space in which surface forces are effective, economy of surface with reference to volume is demanded, within limits. According to Lewis (1925), Graustein's calculations show that if a sphere, an orthic tetrakaidecahedron (with plane faces), and

[3] The terms polyhedra, 13-hedra, 14-hedra, etc., are used for convenience, although the faces were not necessarily plane.

a rhombic dodecahedron all have equal volumes, then the surface of the orthic tetrakaidecahedron is 1.0987 times that of the sphere, and the surface of the rhombic dodecahedron is 1.1050 times that of the sphere.

Using available formulas for the surface and volume of the regular pentagonal dodecahedron, it can be calculated that the surface of this dodecahedron is 1.0984 times that of a sphere of equal volume. While this difference in the fourth decimal place between the tetrakaidecahedron and the regular dodecahedron cannot be of importance in the action of surface forces, it is evident that the regular pentagonal dodecahedron does satisfy the additional condition of surface economy, at least as effectively as the orthic tetrakaidecahedron. All these features —namely, angles between faces, angles between edges, presence of trihedral angles, and surface economy—serve to explain the common occurrence of the pentagonal dodecahedron in a system of bubbles, and to some extent at least, the abundance of pentagonal faces in soap films and in plant cells.

As pointed out above, the average number of contacts of the 600 central bubbles was 13.70. This approximates 14 rather closely. On the other hand, the central bubbles were tabulated in three different sets of experiments, and in each instance the average was less than 14. The average for the 400 peripheral bubbles, as shown above, was 10.99—very close to 11. Lewis (1944) has demonstrated that "for every horizontal layer of 14-hedral parenchymal elements there will be an epidermal layer presenting twelve fewer facets than if they were all 11-hedra." Lewis's "parenchymal elements" correspond to the central bubbles, and his "epidermal layer" to the peripheral bubbles. He has pointed out further that, on a geometrical basis, an increase in the number of faces of epidermal cells must be compensated by a decrease in the facets of the central cells, while an increase in the number of contacts of the central cells would necessitate a decrease in the facets of the epidermal cells. Since the peripheral bubbles here studied had an average of almost precisely 11 faces (10.99), the central bubbles would be expected to average fewer than 14, which they did (13.70).

Comparisons may be made between central bubbles and central lead shot (Marvin, 1939a) compressed in a steel cylinder to eliminate interstices. Marvin found that 624 central shot of uniform diameter had an average of 14.17 contacts, when all "intercellular" spaces were removed in compression. This is fairly close to the average of 13.70 obtained for the 600 central bubbles. The range in contacts in the shot was from 10 to 18, somewhat greater than the range in bubbles, which was from 11 to 17 (table 5). In the shot, as in the bubbles, pentagonal faces were more common than any others.

Although the bubbles in foam and the compressed central shot were similar in certain ways, as shown above, they also displayed significant differences. The range in number of contacts was greater in the shot, and there were fewer shot with 12, 13, and 14 faces. Also in the shot, although pentagonal faces were more numerous than any others, they constituted only 38.7 per cent of the total, and not 66.9 per cent, as in the bubbles.

But the greatest difference between bubbles of uniform volume in a mass and shot of uniform volume compressed to eliminate interstices was in the number of combinations of faces. As shown in table 6, the 600 central bubbles were distributed in 36 different combinations of faces; the 624 central shot (Marvin, 1939a) were arranged in 303 different combinations (Marvin, unpublished table). Of these, 103 combinations could not occur in bubbles because they were modified due to the presence of tetrahedral angles. (There were 144 shot with a total of 159 tetrahedral angles.) All the other 200 combinations found in the shot are geometrically possible in the bubbles; but only 36 combinations occurred in the 600 central bubbles, of which 32 were found also in Marvin's 624 central shot (table 6). When shot or peas are compressed, they assume a large number of different forms, most of which are geometrically possible in the soap films. But upon those geometrically possible combinations an additional factor—surface tension—is superimposed, as a result of which the foam cells assume a relatively few combinations.

A further inspection of tables 6 and 7 shows that the 10 commonest combinations in the bubbles were not the commonest ones in the shot, although all of them occurred in both systems. The four most abundant combinations in the central bubbles (table 7), which include 305 of the total of 600 bubbles, were matched by only 28 of the 624 central shot. The combination of faces in the bubbles found with greatest frequency, 1-10-2 (fig. 30 and table 7), occurred only 5 times in the shot. And while the commonest combination in the bubbles was found 118 times, the most abundant in the shot was found 17 times (2-8-4). In number of combinations, therefore, shot of uniform diameter, compressed in a cylinder, differed markedly from bubbles of uniform diameter in a cylindrical dish.

Marvin (1939a) found 159 tetrahedral angles in the 624 central shot which he studied. In the 600 bubbles analyzed here, there were no tetrahedral angles. This is to be expected, since tetrahedral angles do not occur in soap films, as Plateau (1873) demonstrated.

From these comparisons between data on soap bubbles and on compressed lead shot, it is evident that there is a distinctly greater uniformity in the soap film system. The marked differences between these two types of systems, enumerated above, may be ascribed, to some extent at least, to surface forces, which are more effectively significant in the soap film system than in the lead shot system.

As pointed out in the paragraphs above on periph-

TABLE 8. *Number of faces on cells, compressed lead shot, and bubbles.*

Number of faces	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Average
Elder pith 100 cells Lewis (1923)	1	1	2	0	2	8	8	21	16	19	10	2	3	6	1	0	0	13.97
Human fat 100 cells Lewis (1925)	0	0	0	2	4	5	15	13	20	15	17	4	2	2	1	0	0	14.01
Tadpole precartilage 50 cells Lewis (1933)	0	0	0	1	1	4	9	8	10	7	3	3	2	1	0	1	0	13.90
Eupatorium pith 100 cells Marvin (1939b)	0	1	0	1	7	13	18	14	15	15	7	3	4	1	1	0	0	13.36
Angiopteris petiole parenchyma 100 cells Higinbotham (1942)	0	0	2	2	7	11	15	19	14	7	8	7	4	1	1	0	2	13.55 (13.81)[a]
Citrus carpel vesicles 100 cells Dodd (1944)	0	0	0	2	7	6	12	12	22	18	11	3	7	0	0	0	0	13.85
Ailanthus pith 150 cells Hulbary (1944)	0	0	0	1	7	9	19	22	26	20	21	12	8	5	0	0	0	14.23
Totals 700 cells	1	2	4	9	35	56	96	109	123	101	77	34	30	16	4	1	2	13.86 (13.90)[a]
Total 250 cells Lewis	1	1	2	3	7	17	32	42	46	41	30	9	7	9	2	1	0	13.972
Total 450 cells Columbia	0	1	2	6	28	39	64	67	77	60	47	25	23	7	2	0	2	13.80 (13.86)[a]
624 central shot Marvin (1939a)	0	0	0	0	1	12	50	120	206	139	65	29	2	0	0	0	0	14.17
600 central bubbles	0	0	0	0	0	2	73	179	218	106	20	2	0	0	0	0	0	13.70

[a] Allowing for elimination of intercellular spaces.

eral bubbles, a considerable number of essentially undifferentiated tissues have been studied in recent years. This work has been done in part by Lewis and in part by various investigators at Columbia University. A summary of these results is given in table 8. There is one important circumstance that must be taken into account in any consideration of the results in table 8—namely, that the shot and the bubbles there considered were of uniform size, but the cells, although primarily undifferentiated, varied materially in size. The volume ratios of smallest to largest cells in Marvin's *Eupatorium* pith were 1:15 (1939b) and 1:14 (1944). While these are extremes, they do show that there was a very considerable range in the volume of the cells studied. It might be expected, therefore, that the cells would show greater variation than the compressed shot and than the bubbles thus far studied; in some respects they do. Two figures, 13.55 and 13.81, are given for Higinbotham's results in table 8; the first is the number of contacts actually found; the second, in parentheses, is the number of faces which would have been present if certain intercellular spaces had been eliminated—if "lost contacts" are included.

The 700 cells recorded in table 8 had an average of 13.86 (13.90) contacts (the number in parentheses includes Higinbotham's "lost contacts"); Lewis's 250 cells averaged 13.97, the 450 Columbia cells averaged 13.80 (13.86). The average number of contacts for all the undifferentiated cells studied thus far, 13.86 (13.90), was closer to the average for 600 central bubbles (13.70) than to the average for 624 central shot (14.17). Since Lewis's cells averaged almost precisely 14 contacts—13.972—his average was somewhat closer to the average for the shot, while the 450 Columbia cells—13.80 (13.86)—were distinctly closer to the bubbles. The cells and the bubbles, both chosen at random, were therefore in fairly close agreement, and both had an average of fewer than 14 faces, while in the shot the average was slightly more than 14. The average of the bubbles not including pentagonal dodecahedra—of which none occurred in cells—was 13.86—precisely the same as that for the 700 cells in table 8, and the same as the average of the Columbia cells counting in Higinbotham's "lost contacts."

It is immediately evident from table 8 that the range in number of contacts was distinctly greater in the cells than in the shot and the bubbles. This can be accounted for, at least in part, by variation in volume of the cells (Marvin, 1939b, 1944), while the shot and bubbles were of uniform volume. It is known quite definitely that varying the size of the shot increases the range in the number of contacts (Matzke, 1939). The same is true for bubbles and cells. This greater range in number of contacts in the cells is, therefore, to be expected.

TABLE 9. *Number of sides per face of cells, compressed lead shot, and bubbles.*

Number of sides per face	2	3	4	5	6	7	8	9	Total number of faces
Frequency									
Eupatorium pith 100 cells Marvin (1939b)	0	4	418	471	363	71	7	2	1336
Angiopteris petiole parenchyma 100 cells Higinbotham (1942)	0	11	352	596	291	94	10	1	1355
Citrus carpel vesicles 100 cells Dodd (1944)	0	11	359	576	327	97	15	0	1385
Ailanthus pith 150 cells Hulbary (1944)	0	6	565	822	594	128	18	2	2135
Totals									
450 cells[a]	0 (0)	32 (42.4)	1694 (2242.2)	2465 (3262.7)	1575 (2084.7)	390 (516.2)	50 (66.2)	5 (6.6)	6211
624 central shot[a] Marvin (1939a)	1 (.93)	465 (432.4)	1921 (1786.5)	3420 (3180.5)	2276 (2116.6)	666 (619.4)	84 (78.1)	7 (6.5)	8840
600 central bubbles	0	0	866	5503	1817	35	0	0	8221

[a] Since 8221 faces were tabulated in the bubbles, 8840 in the shot, and 6211 in the cells, comparable values for the shot and the cells are given in parentheses.

In table 9 the numbers of triangular, quadrilateral, pentagonal, etc., faces in the 450 Columbia cells, in the central shot of Marvin (1939a), and in the central bubbles are given. Since 600 bubbles, with a total of 8221 faces, were tabulated, and 624 shot with 8840 faces, and 450 cells with 6211 faces, the values for the shot and for the cells which are comparable with the bubbles are given in parentheses below each number. In the cells, shot, and bubbles, there were always more pentagonal faces than any other kind. However, there were more pentagonal faces in the bubbles than all other kinds added together (66.9 per cent were pentagonal). In the cells a slightly greater proportion of the total faces (39.7 per cent) was pentagonal than in the shot (38.7 per cent). It will be recalled that, according to Plateau's (1873) calculations, angles between edges should be 109°28′16″, and that the angles of a regular pentagon are 108°; they therefore require but slight modification to satisfy this condition. Such angles may become modified by curvatures of the edges and of the films. It is not surprising, then, that pentagonal faces were found so very abundantly in soap film systems. That the cells did not approach the bubbles more closely can be explained, in part at least, by the variation in volume of the cells. If bubbles of varying sizes are mixed together, the proportion of pentagonal faces is reduced (unpublished data). Although the cells varied considerably in volume, they still had a fairly high proportion of pentagonal faces.

It is further obvious from table 9 that there were no triangular faces in the central bubbles, no octagonal and no nonagonal faces. Triangular faces, which did not occur in the central bubbles, were abundant in the central shot (465) and rare in the cells (32). The angles of an equilateral triangle are 60°, and therefore quite different from the required 109°28′16″. Heptagonal faces, which were rare in the bubbles (35), were found in both shot and cells, but definitely in greater frequency in the shot. Similarly, octagonal faces, which did not occur in the bubbles, were more numerous in the compressed shot than in the cells. If bubbles of varying volume are mixed together, the number of heptagonal, octagonal, and nonagonal faces is increased, approaching more closely the numbers in cells (unpublished data). In all these respects the cells were intermediate between the bubbles and the shot, suggesting quite definitely that surface forces were significant in the cells. The evidence here, as elsewhere, indicates that surface forces constitute one of the factors—but not the only one by any means—important in cell shape determination.[4]

As shown in table 6, the 600 bubbles occurred in 36 different combinations of faces. Marvin's (1939a) central shot were arranged in 303 different combinations; and 550 undifferentiated cells (Marvin, 1939b, 1944; Higinbotham, 1942; Dodd, 1944; Hulbary, 1944) were found in 203 different combinations. Here again there were many more combinations in the cells than in the bubbles, but not nearly so many as in the shot. This was in spite of

[4] Professor F. T. Lewis and Professor J. W. Marvin carefully scrutinized the manuscript, as prepublication reviewers for the American Journal of Botany. For the many constructive suggestions which they offered I am very grateful. They both noted that in the foam cells the faces were more nearly equal in area than in compressed shot or than in plant and animal cells. This had already been pointed out in a second manuscript, which was submitted for publication before the reviewers' comments were received. No statistical data are available on the area of the faces.

ıe fact that the shot and the bubbles were of uni-ɔrm volume, while the cell volumes varied greatly. 'hen shot of varying diameters were mixed and ıen compressed, the number of combinations was aterially increased (Matzke, 1939, unpublished ble); similarly, when small and large soap bub-es were mixed, the number of combinations of ces rose sharply (unpublished data). Notwith-anding these facts, the undifferentiated cells, hich varied greatly in volume, showed distinctly wer combinations than the central shot (Marvin,)39a), which were of constant volume, and they ıowed far fewer combinations than shot of vary-g volumes (Matzke, 1939). From this viewpoint ıe cells were again intermediate between the bub-es on the one hand and the shot on the other.

Approaching the same question from still an-her standpoint, there were 192 cells (out of 550) hich matched the 36 combinations found in the)0 central bubbles (table 6); there were 315 cells hich matched the 303 combinations of the 624 cen-al shot of Marvin. At first glance, it might seem ıat the cells approached the shot more closely than ıe bubbles in this respect. However, it is hardly ıstifiable to match the 36 combinations in the bub-es against the 303 in the shot in this way. If, on ıe other hand, the 36 most common combinations the 624 shot of Marvin are compared with the) combinations in the bubbles, there are 158 cells hich match the 36 most frequent shot combina-ons, compared with 192 cells which match the 36 ıbble combinations. Therefore, 35 per cent of the 50 cells are distributed in 15 per cent of the total f 203 cell combinations—the 15 per cent which ave the same combinations as the central bubbles. onsidered from this angle, the cells correspond ıore closely to the bubbles in foam than to the shot ɔmpressed to eliminate interstices. Once more a ystem in which surface forces are of controlling nportance approaches the cells more closely than ne in which they are not.

From tables 6 and 7 it is evident that the com-ınations which were of greatest frequency in the ıubbles were not necessarily the ones which were ıost common in the cells or in the shot. Thus the 5 ıost abundant combinations in the bubbles (table ') were found sparingly or not at all in the cells. 'or instance, no undifferentiated cell has been found ıs yet to match the pentagonal dodecahedron. The ɔombination 3-6-4, however, which was the sixth ıost abundant in the bubbles (table 7), was the ɔne of greatest frequency in the undifferentiated ells, and the second most common in the shot. In general, however, as outlined in tables 6 and 7, the ɔommonest combinations in the bubbles were not he same as the ones of greatest frequency in the ells or in the shot. This may be explained in part, gain, by the unequal volume of the cells, as con-rasted with the uniform volume of the bubbles. It strongly suggests that surface forces are not alone responsible for cell shape determination, but that other factors must also be taken into consideration.

As Plateau pointed out, more than three film surfaces do not meet in a point in one foam cell. On the other hand, individual compressed shot and individual cells not uncommonly have four faces meeting in a point, forming "tetrahedral" angles. In 624 central shot, Marvin (1939a) recorded 159 such tetrahedral angles. If surface forces were not of distinct importance in the cells, the 550 undifferentiated cells might be expected to have approximately 140 tetrahedral angles. Actually, however, only 30 such angles have been found in the 550 cells. Of course, none were seen in the 600 central bubbles. When large and small shot were mixed, 39 tetrahedral angles were found on 300 shot (Matzke, 1939). While these systems again are not perfectly comparable, due to differences in magnifications necessarily used and to size variations in the cells, the conclusion once more seems inescapable that the cells are intermediate between liquid and non-liquid systems.

Various investigators have emphasized factors that may be concerned in the determination of the three-dimensional shapes of cells. Many years ago Harper (1916) pointed out that cells in a colony of the alga *Pediastrum* adjust themselves as a result of mutual pressure, adhesion, surface tension, and inherited form tendencies. In presenting the tetrakaidecahedron of Kelvin as a possible shape for cells in tissues, Thompson (1917) in effect was suggesting a semi-regular, least-surface configuration. Recently Lewis (1943a), after a long series of investigations, reemphasized the tetrakaidecahedron, but an orthoid one, having, like Kelvin's, 8 hexagonal and 6 quadrilateral faces, but lacking the regularity of Kelvin's figure. In speaking of cells, Lewis states that "Notwithstanding the rarity of its occurrence, the orthoid tetrakaidecahedron is the form about which the observed shapes hover." ". . . . it may properly be regarded as the typical shape of cells in masses. There is no rival pattern." In his most recent publication Lewis (1944) places particular emphasis upon the "necessities of spherical geometry, acting upon bodies regardless of their surface tension." That the necessities of spherical geometry are basic certainly cannot be denied. But within the confines of spherical geometry there is a range within which other forces are operative and which tend to impose further limitations. Bounded by such limitations, development may occur.

In his numerous publications Lewis has shown how various combinations of faces in cells can be derived from the tetrakaidecahedra of Kelvin. While the combinations described above for the bubbles are derivable from Kelvin's tetrakaidecahedra, there is no evidence that the foam cells, which at first are spherical, become changed into the Kelvin figures, and are then further modified into the shapes and combinations of faces listed and figured above. In a sense, Kelvin's figures may be considered as the "ideal type," but they are not realized

in soap films under the conditions here portrayed.

That surface forces are present in protoplasm is no new concept. Their significance in protoplasmic structure and in cell division is discussed by numerous authors, including Bütschli (1900), McClendon (1910), and Gortner (1938). This does not mean that they are the controlling forces, or necessarily the most important ones in development. It means that they are there, and, unless they are overcome, they may be expected to operate. This is evident from Robert's work (1902) and from the more recent investigations of a number of other authors. The tension at the surface of eggs of *Chaetopterus* has been measured (Harvey, 1931a), as has the tension at the surface of eggs of the sea urchin *Arbacia* (Harvey, 1931b), at the surface of *Amoeba dubia* (Harvey and Marsland, 1932), and at the surface of the slime mould *Physarum polycephalum* (Vexler, 1935). Harvey and Schoepfle (1939) have also measured the interfacial tension of intracellular oil drops in the eggs of *Daphnia pulex* and in *Amoeba proteus*. In general these tensions, which are not necessarily surface tensions alone, are low, often of the order of one dyne per centimeter. Significant in this connection also is the work of Marsland (1938, 1942), who subjected fertilized eggs of *Arbacia punctulata,* in which cleavage furrows had appeared, to pressures of 450 atmospheres. This pressure "is sufficient to induce a marked liquefaction of the protoplasm of the egg cortex, which normally at the time of cleavage exhibits the properties of a firm gel." Marsland found that the progress of the furrow ceases, a slow recession commences, and, if the pressure is maintained, the eggs become "almost spherical again." This work suggests the importance of the sol-gel condition and its part in the overcoming of surface forces in cleaving eggs. "According to this view the cells become rounded under the agency of surface forces as soon as, due to the solating effect of the pressure, the resistance of the plasmagel drops below a certain critical value." This is linked with the general problem of viscosity changes in cells. Increase and decrease in viscosity have been studied fairly extensively in dividing cells by Chambers (1917), Heilbrunn (1921, 1943), Zimmerman (1923), and Kostoff (1930).

Anyone who considers carefully the part that interfacial tensions may play in three-dimensional cell shape determination will realize that they are not the only instrumental factors. The increase in length of certain plant cells is obviously in violation of the principle of least surfaces, as is the process of cleavage. The importance of surface forces has been questioned by Sinnott and Bloch (1941a, 1941b) in certain types of dividing cells; their contentions might seem to be more especially applicable "in tissues where cell division had been induced by wounding," and in "relatively slowly growing or thick-walled cells," though they suggest a wider validity.

In the paragraphs above, comparisons have been drawn between the three-dimensional shape of measured bubbles in foam, cells in undifferentiated tissues, and lead shot of uniform diameters compressed to eliminate interstices. In the bubbles in foam the compartments are bounded by liquid films in which surface tension is definitely significant; in the lead shot surface forces, while present, are not of controlling importance. Analyzed from many different viewpoints, the cells have been shown to be intermediate between the bubbles and the compressed shot, suggesting that surface forces play a part when cell shapes are determined, though these forces are not so free to operate as in the case of the bubbles in foam. The comparisons have been made especially with undifferentiated cells. This is not to be construed as meaning that interfacial tensions are the sole or necessarily the most important of all the factors responsible for three-dimensional cell shape determination. Furthermore, it does not mean that they are always significant in cellular development. On the other hand, meristematic cells in plants are often liquid or semi-liquid, and when the new walls are first formed, they are exceedingly delicate. It is logical to think that interfacial tensions may play a part in the fine adjustments responsible for the shapes of the cells in meristems, even though these same cells may have hard, stiff walls later on. The evidence presented above indicates that interfacial tensions are important in the determination of three-dimensional cell shapes.

SUMMARY

A uniform foam was constructed by placing bubbles made of a special soap solution, one by one, into a cylindrical dish. The volume of each bubble was controlled by setting the plunger of a Yale tuberculin syringe at 1/10 (or 1/20) of a cc., and making a bubble with the enclosed air. In this way a dish was filled with nearly 2000 (or 4000) bubbles, each of the same controlled volume. Then with a dissecting microscope it was possible to single out, by focusing, any bubble in such a mass and record with precision the number and kinds of its faces. The exact three-dimensional shape of bubbles in foam has been for many years a subject of discussion and speculation among mathematical physicists (Plateau, Kelvin), metallurgists, plant and animal histologists, and students of protoplasmic structure.

Studied in this way, 400 peripheral (epidermal) bubbles, of which 200 were free and 200 in contact with the walls of the cylinder, averaged 10.99 faces, with a range from 7 to 14. More than half of the faces of the peripheral bubbles were pentagonal, and 98 per cent were quadrilateral, pentagonal, or hexagonal. No one combination of faces among those found strongly predominated or could be considered "the type." The commonest of the 43 combinations of faces which were found had 11 faces,

of which 3 were quadrilateral, 6 pentagonal, and 2 hexagonal; there was normally one hexagonal face above, in addition to 6 lateral contacts and 4 basal ones. This combination occurred 67 times and constituted 17 per cent of the total number studied. Pentagonal dodecahedra occurred 11 times among the 400 peripheral bubbles.

Comparisons were made between peripheral bubbles in foam, peripheral compressed lead shot, and cells in tissues. The bubbles were more uniform than the shot or than the cells. The cells showed a much closer resemblance to the peripheral bubbles than to the peripheral shot, suggesting strongly that surface forces, which are of relatively greater importance in the bubbles than in the shot, are also distinctly significant when cell shapes are being determined.

The average number of faces, 10.99, agreed with 11-faceted epidermal cells previously reported by Lewis.

The average number of contacts of 600 central bubbles was 13.70, the range being from 11 to 17. More than a third (218) of the 600 foam cells had 14 faces, and more than five-sixths had 13, 14, or 15 contacts. More than two-thirds of all the faces were pentagonal, and 99.6 per cent of all the faces were quadrilateral, pentagonal, and hexagonal.

The 600 central bubbles tabulated occurred in 36 different combinations of faces. As in the peripheral bubbles, there was no one combination of those occurring that was strongly preponderant and that could be considered as "the type." The most abundant combination was a 13-faceted foam cell with 1 quadrilateral, 10 pentagonal, and 2 hexagonal faces (1-10-2) which occurred 118 times among the 600 bubbles. The second and third most frequent combinations were of bubbles with 14 faces, 1-10-3 and 2-8-4, which were found 73 and 64 times, respectively. The pentagonal dodecahedron, with 12 faces all pentagons, occurred 50 times. These 4 combinations accounted for 305 of the 600 bubbles.

When a large number of bubbles, all of equal volume, are placed into a cylindrical dish so that they are free to glide and adjust themselves, they do not assume the form of the orthic or of the minimal tetrakaidecahedra of Lord Kelvin. Instead, they become arranged in a mass of somewhat irregular foam cells, satisfying, in approximation at least, the equilibrium conditions of Plateau. In this system pentagonal faces predominate; consequently the foam cells, although averaging close to 14 faces as in Kelvin's tetrakaidecahedra, differ from Kelvin's figures, since the latter have all hexagonal and quadrilateral faces. In the foam pentagonal dodecahedra, in which all the faces are pentagons, are fairly common, and it is demonstrated that this figure requires but little modification to satisfy the equilibrium conditions for angles as specified by Plateau. Calculation shows further that the pentagonal dodecahedron is slightly more economical in surface per unit of volume than the orthic tetrakaidecahedron and than the rhombic dodecahedron.

Comparisons were made between central bubbles and central lead shot compressed in a steel cylinder to eliminate interstices. The average number of contacts and the range in number of contacts were slightly less in the bubbles than in the shot. The number of pentagonal faces was much greater in the bubbles than in the shot. The 600 central bubbles occurred in 36 different combinations of faces, while 624 central shot of Marvin occurred in 303 combinations. The commonest combinations in the bubbles were not the most abundant ones in the shot. There were no tetrahedral angles in the bubbles, and they were frequent in the shot. While there were certain similarities between compressed shot and bubbles in foam, there were also characteristic differences, which could be ascribed, in part at least, to the surface forces in the soap film system.

Detailed comparisons were also drawn between central bubbles in foam and cells in essentially undifferentiated tissues; and they were both compared with the compressed lead shot. In average number of contacts the cells were intermediate between bubbles and shot, but closer to the bubbles. In number of pentagonal, triangular, heptagonal and octagonal faces the cells again stood between bubbles and compressed lead shot. Similarly, the number of combinations of faces was least in the bubbles, and greatest in the shot, while the cells were again intermediate. Tetrahedral angles occurred abundantly in the shot, less frequently in the cells, and not at all in the soap film system.

Since cells are intermediate in so many ways between the soap bubbles, in which surface forces are of controlling importance, and the compressed lead shot, in which they are not, and since the cells show a much closer similarity to bubbles in foam than to compressed shot, the conclusion is drawn that interfacial forces constitute one of the factors determining the shape of essentially undifferentiated cells at or near the plant meristems. This is not to be construed as meaning that interfacial tensions are the only or necessarily the most significant factor. The evidence here assembled indicates that surface forces are important.

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A STUDY OF THE STOMATE IN SUGARCANE [1]

Lewis H. Flint and Charles F. Moreland

INVESTIGATIONS OF the red rot of sugarcane in these laboratories have included researches on hyphal penetration of the epidermal cells of sugarcane leaves and of fungus fruiting structures occupying the cells of the stomatal region or the enclosed orifice. In the course of these studies, it became evident that the sugarcane stomate was appreciably more differentiated than those of the grasses in general. A survey of the literature failed to reveal any description of such complexities or of the nuclear behavior in the guard cells in the later stages of development, and a further study of the sugarcane stomate has yielded the results herein presented.

The researches of Campbell (1881) on the development of the stomate of *Tradescantia* and of Indian corn were outstanding. In both plants he observed the formation of two small accessory or auxiliary cells from the epidermal cells adjoining the stomate-mother-cell, and the early development of the guard cells. At the stage in which the guard cells became bean-shaped, the auxiliary cells were still inconspicuous. Later stages involved varying degrees of median constriction of the guard cells. These early stages were described also by Porterfield (1937) in bamboo.

Schwendener (1899) described the variety of form present in the stomates of grasses and sedges. Emphasis was placed upon shape and mode of action of the guard cells. No references were made to the nuclear condition within the guard cells.

MATERIALS AND METHODS.—The following varieties of sugarcane were used: Louisiana Purple, Louisiana Striped, Creole, D. 74, D. 95, L. 511, F. 31–962, P.O.J. 234, Co. 281, Co. 290, C.P. 33/243, C.P. 29/320, and C.P. 36/55. For the most part, material fresh from the field was studied by means of free-hand sections. The material selected for study ranged from soft tissues, in which the stomates were first differentiated, to the mature tissues of the sheath and blade. Critical aspects of nuclear behavior were studied with the aid of microtome sections and the Feulgen stain.

RESULTS.—The patterns of stomate distribution differed somewhat in different varieties of sugarcane, and there were minor differences in stomatal structure in different varieties. In general, however, the range of variation between varieties was less than that within a given variety. Such factors as position, rate of growth, turgidity of tissue, exposure to light, etc., resulted in greater variation than that between varieties. It was considered sufficiently accurate, therefore, to indicate the general trend of developments.

The early development of the stomate in sugarcane was found to follow the pattern described for *Tradescantia* and *Zea* by Campbell to the attainment of the stage characterized by bean-shaped guard cells with lenticular auxiliary cells. Stages in this pattern included the stomate mother cell, the mother cell with two lateral auxiliary cells, the mother cell in division and the stomate with bean-shaped guard cells and lenticular auxiliary cells. These stages have been shown respectively in figures 1 to 5 (drawings). Some of these stages have also been shown in figures 14 and 15 (photographs).

The later development of the stomate in sugar-

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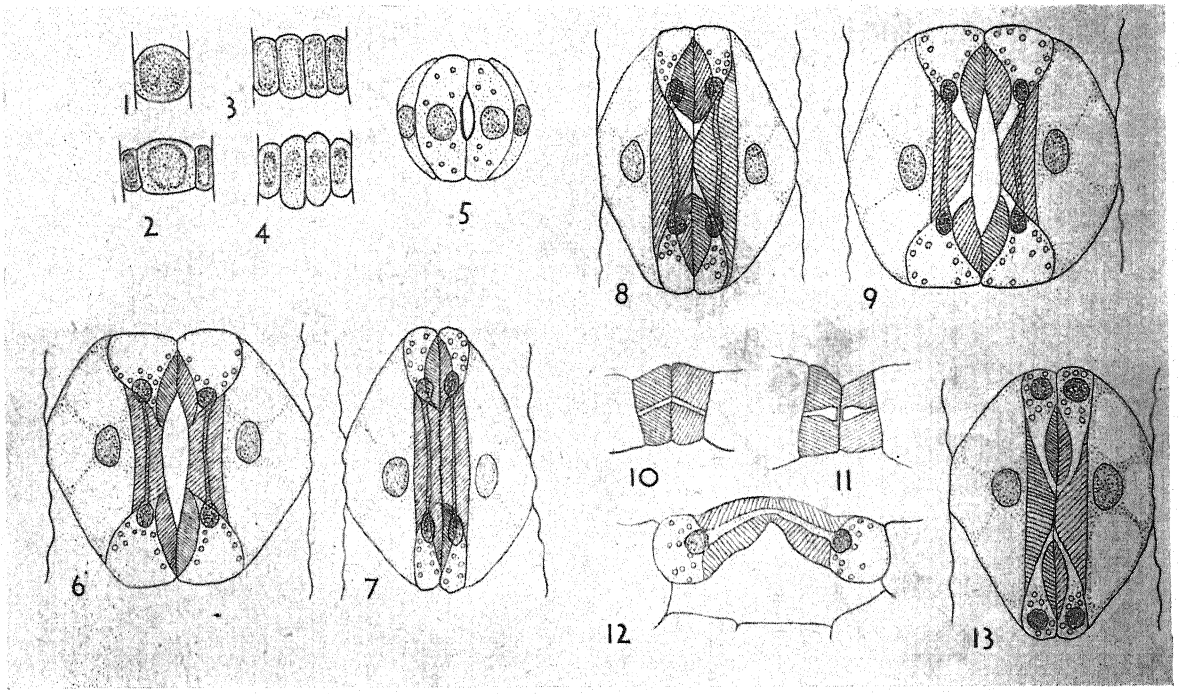


Fig. 1–13.—Fig. 1. The stomate mother-cell.—Fig. 2. The stomate mother-cell with auxiliary cells developed from adjoining cells.—Fig. 3 and 4. Successive early stages of guard-cell development.—Fig. 5. The simple-type stomate with bean-shaped guard cells and compressed auxiliary cells.—Fig. 6 and 7. Guard-cell, external surface view, open and closed.—Fig. 8 and 9. Guard-cell, internal surface view, closed and open.—Fig. 10 and 11. Cross-sections through central portion of guard cells.—Fig. 12. Longi-section of one guard cell.—Fig. 13. Stomate with two apparently-independent nuclei in each guard cell.

cane involves extensive modifications in the shape and wall structure of the guard cells. The thin-walled bean-shaped guard cells become almost straight. The nuclei of these cells are slightly elongated, their shape being somewhat correlated with the shape of the guard cells. Later the guard cells take on the typical dumb-bell shape by an apparent constriction of their median parts and an enlargement of their ends. When the guard cells have attained their full size, the elaboration of an intricate system of wall-thickenings takes place. These thickenings take place rapidly and are so extensive as to reduce greatly the lumen in the median parts of the guard cells. Associated with this progressive constriction is the development of a dumb-bell shaped nucleus in which most of the chromatic material is contained within the bulbous ends, which are connected by a very narrow thread of nuclear material. Simultaneously with the development of the wall-thickenings and the attainment of a dumb-bell-shaped nucleus, there is a marked increase in the size of the auxiliary cells. These later developments have been indicated in figures 6 to 12, showing views of mature stomates from the outside, open and closed (fig. 6 and 7); from within, closed and open (fig. 8 and 9); in cross-section (fig. 10 and 11); and in longi-section (fig. 12). The wall thickenings have been indicated by cross hatching.

While the developments as above outlined appeared to characterize the mature sugarcane stomate, in some instances a further separation of the knob-like chromatic terminals of the dumb-bell-shaped nuclei was observed, to yield two nuclei in each guard cell. In some cases these nuclei were smaller than the nuclei of adjacent cells while in other cases they had attained a normal size. In figure 16 there is shown a stage in which the knob-like chromatic terminals are connected only by a thin skein of protoplasmic material. In figures 13 and 17 a stage is shown representing further developments in which two spherical nuclei are present in each guard cell. Notwithstanding these developments, transverse and longitudinal sections of the stomates have consistently revealed that there is no closing of the passageway connecting the two bulbous terminal ends of the guard cells.

Discussion.—One of the outstanding features of the sugarcane stomate as compared with the stomates more typical of grasses is the extensive and intricate system of thickenings in the walls of the central parts of guard cells. In mature stomates the lumen of the median part of the guard cell is reduced to a mere slit. Another feature, which is associated with this extensive thickening of the guard cells, is the fact that the progressive constriction appears appreciably to isolate the opposite

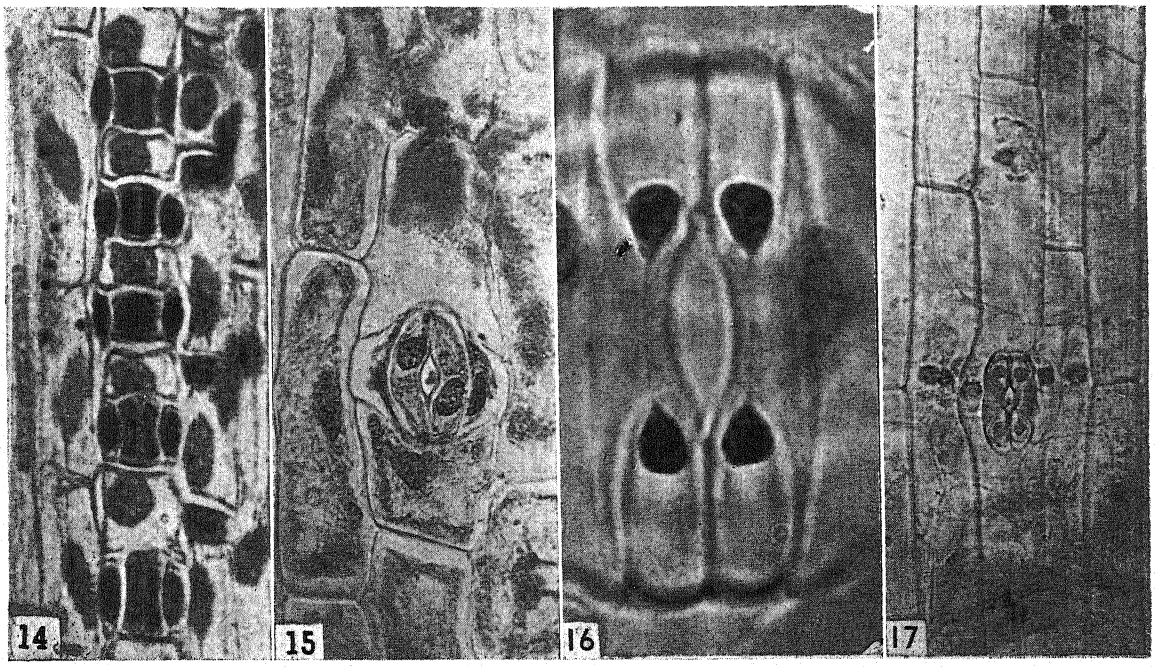


Fig. 14–17.—Fig. 14. Stomate mother cells, including some in division to form guard cells. ×780.—Fig. 15. The simple-type stomate with bean-shaped guard cells. ×390.—Fig. 16. The dumbbell type nucleus commonly present in the guard cells of sugarcane stomates. ×1850.—Fig. 17. Development involving two apparently-independent nuclei in the guard-cells of a sugarcane stomate. ×390.

terminals of the guard cells, in the course of which the knob-like chromatic terminals of the guard cell nuclei appear to attain an increasing degree of independence. In extreme cases a complete separation of the knob-like terminals of the nuclei was seen, followed by the development of a nuclear membrane around each terminal to yield two spherical nuclei in each guard cell.

Because of these developments, further study was directed to the manner of separation of the chromatic material in the nucleus, i.e., whether it is mitotic or amitotic. While in a few instances, particularly in sheath tissue, a somewhat orderly alignment and separation of more or less uniform chromatic granules, suggesting mitosis, was observed, separation in general appeared to involve heterogeneous chromatic granules disengaged in a disorderly manner, suggesting amitosis.

SUMMARY

In sugarcane the early development of the stomate is in general agreement with that characteristic of grasses; the later stages of development involve extensive and intricate thickenings of portions of the walls of the guard cells.

Simultaneous with the extensive thickening of the walls of the central part of the guard cell, a separation of the chromatic material of the guard cell nucleus occurs with the result that the nuclear knobs in the terminal sacks of the guard cells are connected only by a very fine thread of nuclear material which extends through the narrow central part.

Under extreme conditions the knob-like terminals of the dumb-bell-shaped nuclei become so greatly separated that separate spherical nuclei are formed, one in each terminal part of the guard cell. While in some instances these nuclei are smaller than those of adjoining single-nucleated cells, in other instances they are of normal size.

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BOTANICAL SURVEY OF THE ANGEL MOUNDS SITE, EVANSVILLE, INDIANA [1]

Helen Marsh Zeiner

AN UNUSUAL opportunity to correlate botanical and archaeological studies arose when it was observed by archaeologists at the Angel Mounds on the Ohio River east of Evansville, Indiana, that the course of the wall of the old Indian village and the locations of earthworks could be traced by the distribution of certain species of plants, although the fields in which the site is located have been under cultivation for many years. This plant distribution is shown in an especially striking way in aerial photographs.

In an effort to discover a possible explanation for the plant differences, a detailed ecological study of the site of the wall was undertaken. In addition to this, a general survey of the vascular plants occurring on the site was made to round out the picture. It is the purpose of this paper to present the results of these studies.

THE ANGEL MOUNDS SITE.—The Angel Mounds site, about seven and one-half miles east and south of Evansville, Indiana, is an area of 450 acres lying along the northern bank of the Ohio River in the extreme eastern edge of Vanderburgh County and

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The writer wishes to express her appreciation for many helpful suggestions and for constant encouragement to Dr. Paul Weatherwax, under whose direction this study was undertaken. She also wishes to thank the Indiana Historical Society for the financial support which made the study possible, and Mr. Glenn Black, director of archaeological research at the Angel Mounds Site, for acquainting her with the archaeological background and problems of the site and for his willing cooperation in helping in every way possible with the study.

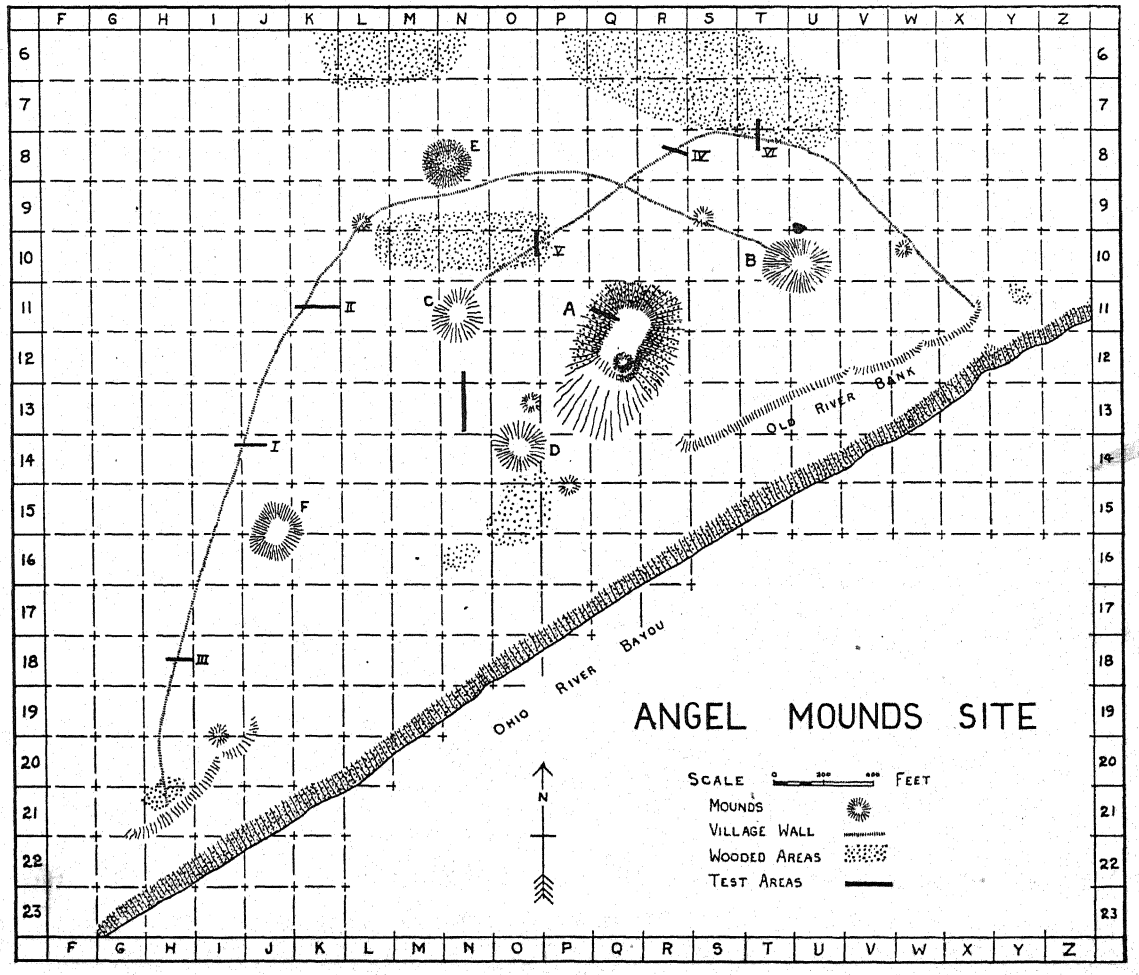


Fig. 1. Map of Angel Mounds Site. Adapted from Lilly, Prehistoric Antiquities of Indiana.

including also a narrow strip along the western margin of Warrick County. An area of about 100 acres of this tract is considered as the "archaeological zone." The botanical survey covered at least 150 acres, of which the archaeological zone was a part.

On approaching the site, one sees slightly rolling fields interrupted by small patches of woods and interspersed with peculiarly symmetrical hills, the mounds, of which there are eight of an obvious nature and others more or less questionable (fig. 1). The largest and most striking (Mound A) is a rectangular structure 31 feet high and about 520 by 150 feet at the base, with a flat top 100 feet by 200 feet. A lower ramp slopes southward 100 feet into the fields. At the southeast corner of the flat top, there is a conical addition about 14 feet high. Since the sides of the mound are so steep that they cannot well be cultivated, they have been allowed to grow up to trees; but the flat top and the ramp have long been cultivated. Mounds E and F, both large conical structures, are also impressive in size. Mound F has been excavated in connection with an extensive study of the site from an archaeological viewpoint which is being carried out by the Indiana Historical Society under the direction of Mr. Glenn Black. A report on the progress of the archaeological research will be found in the *Proceedings of the Indiana Academy of Science* for 1941.

The tract is a part of an extensive natural terrace built up by deposits of lacustrine silts during the Wisconsin glacial epoch. During this period, the Wabash River carried such an enormous volume of water that its tributaries were ponded, and even the Ohio and its tributaries were dammed back, forming expansive lakes in southern Indiana and Kentucky (Thornbury, 1937, p. 69). As the glacier receded and the ponded waters drained, the terraces of lake silts which had been laid down in them were exposed.

The soil of the terrace is a very fine yellowish brown clay silt, which has been described as texturally much the same as true loess, or wind-blown soil deposits.

The area as it stands today comprises several different habitats or ecological areas. These may be classed roughly as open fallow fields and wooded areas, including dry woods, swampy woods, wooded river bank, and wooded mounds.

The main part of the area under survey is composed of fallow fields. Most of these are mowed at some time during the summer to keep the weeds under control. One large field, however, was left unmowed, and had already begun to show a good stand of scrub sassafras (*Sassafras albidum* (Nutt.) Nees) and other woody plants.

The dry woodland is made up of a mixture of hardwood trees, in which *Quercus falcata* Michx., *Q. velutina* Lam., *Carya ovata* (Miller) Koch, *C. cordiformis* (Wanghm.) Koch, and *Acer saccharum* Marsh. are among the leading species, with a dense undergrowth of trumpet creeper (*Campsis radicans* (L.) Seemann). There are few wild flowers, and of these the majority are early spring bloomers, such as *Viola* spp., *Galium aparine* L., and *Ranunculus abortivus* L. The wet woodland is situated along an abandoned stream bed in which water completely covers the ground during the spring months, but which becomes very dry during the summer. This woodland is also made up of a variety of trees, with many swamp cottonwoods (*Populus heterophylla* L.) in the wettest portions. In these parts is found an undergrowth of mallow (*Hibiscus militaris* Cav.), buttonbush (*Cephalanthus occidentalis var. pubescens* Raf.), and water-loving plants such as the cardinal flower (*Lobelia cardinalis* L.). In the drier parts of the woods there is little undergrowth.

The river-bank is subject to spring floods, but surface water drains away quickly, soon leaving the soil dry and cracked. The trees are largely sycamores (*Platanus occidentalis* L.), willows (*Salix* spp.), and the soft maples (*Acer negundo* L., *A. rubrum* L., and *A. saccharinum* L.). There is less undergrowth than in the wet woods, but the species are similar.

The wooded mounds resemble the dry woods. There is an abundance of early spring flowers, particularly on the largest mound, Mound A, and a lesser number of blooming plants during the summer and autumn.

The archaeological studies indicate that the inhabitants of the Angel site were a southeastern people who made a northern migration, settled Angel site, occupied it for an undetermined period of time, and then left. The causes of the migration and subsequent abandonment of the site are still in a speculative state.

It is known that these aborigines were an agricultural people living in an extensive village protected on three sides by a log palisade and on the fourth by the Ohio River. The walls of their houses were of logs placed vertically in trenches and lathed with cane over which mud and straw were plastered. The roofs were thatched with grass, with an opening in the top to let out smoke from interior fires. The village must have bustled with activity, for, besides cultivating corn, these Indians fished, hunted, made pottery and implements, wove textiles, and carried out numerous other activities. The mounds represent a tremendous amount of labor; as excavation of Mound F shows, they were built up of small loads of soil, such as might have been carried in baskets. When the size of the mounds is compared with the size of a load which could have been carried by an Indian worker, the enormity of the task becomes impressive.

When the Angel Mounds inhabitants abandoned the site, they left behind them a record which has persisted through the years. To the archaeologist this is rich with meaning; but there is much to interest other groups. To the botanist, the problem

of the wall site and the few evidences of plant remains have special appeal.

GENERAL TAXONOMIC SURVEY.—A general taxonomic survey of the vascular plants occurring on the site was begun early in the spring of 1941, the first collection being made on April 12. Weekly or bi-weekly visits, depending on conditions of plants and weather, were made until the plants were killed by frost in the autumn. The following summer the same procedure was followed.

During the two seasons, 339 different species of vascular plants were collected and identified.[2] Six species which could not be identified beyond the genus were also collected. Of the identified species, 241 represented new records for Vanderburgh County, many of these being very common plants. This reflects the fact that the county has been neglected by plant collectors. The survey did, however, yield some plants rare enough to be worthy of special mention.

Perhaps of most interest was *Hottonia inflata* Ell. (Water Milfoil) which had been previously collected only three times in Indiana (Deam, 1940, pp. 745–746). The Angel site specimen was collected on May 17, 1941, in a low, wet woods, where eight to ten plants were found in bloom. Although careful watch was kept during the second summer, the plant was not found in any new location, and, unfortunately, the spot where the 1941 collection was made was completely under water until long past the blooming period.

Pyrrhopappus carolinianus (Walt.) DC., for which there is only one previous authentic report for Indiana (Deam, 1940, p. 1013), occurred abundantly at the site. Only a few specimens were found in an open field in 1941, but the following season many specimens were found throughout the fields.

Orobanche ludoviciana Nutt. *var. genuina* G. Beck was found attached to the roots of *Ambrosia trifida* L., along the clay banks of the river slough. The plant had been previously reported from only three counties in the state (Deam, 1940, p. 861), but has since been reported from a fourth county (Guard and Silver, 1942).

The large number of species growing in a comparatively small area calls attention to the possibilities for collecting if one is willing to concentrate on one locale, and points out that it is not necessary to range far afield to find an abundance of varied plant life.

USE OF PLANT MATERIALS BY THE INHABITANTS OF ANGEL SITE.[3]—A large number of the plants growing at Angel Mounds site are indigenous to the state of Indiana, and may, therefore, presumably have been present at the time of occupation. Some evidence of the use of native plant material in this locality has been preserved, but it is slight; if, however, we are to judge by the uses made of native plants by Indians in historic times, the occupants of Angel site undoubtedly made much more use of materials at hand than preserved evidence would indicate.

There is no question that the inhabitants of the site were an agricultural people, as evidenced by the quantities of maize found in the ruins, together with agricultural implements, such as hoes made from the fresh-water unio, and flint or chert spades. A few beans, one a lima bean, occurred in the ruins, indicating that beans were also grown. It is possible that squashes were grown, although no remains have been found to date. This may be due to the perishable qualities of the seeds, which would not lend themselves to preservation in the soil as readily as either maize or beans. While maize was the principal vegetable food of these people, they undoubtedly varied their diet with whatever plant food was available. Wild fruits and nuts are the most obvious addition to their diet, and pecan and hickory nuts were found in the ruins; but any edible plant probably was used at one time or another, especially in time of crop failure or food shortage.

Impressions on potsherds show that the inhabitants made textiles by plaiting, twining, open work, knotted netting, and plaiting in cane. It also appears that they manufactured a cord net, presumably used in fishing. Native plants were, no doubt, the source of the fibers used for textiles and cordage.

Mud daub from house walls shows impressions of grass used with mud in plastering the sides of the houses. *Arundinaria* and a species of *Andropogon* were used in this way. Swatches of grass found in the ruins of the houses indicate that grass was also used for thatched roofs.

Logs were used as house frames and for the palisade wall, and small post holes within the walls of houses apparently indicate the use of smaller logs for making various furnishings such as benches.

Native plants were probably used for many other things, among which may be mentioned medicines, charms, and sources of fuel.

STUDY OF THE WALL SITE.—It was early noted by farmers living near Angel Mounds site that a variation in plants and a somewhat lighter colored soil formed a striking pattern extending in a line across the fields. To find different species of plants occurring in scattered patches in fallow fields is normal and to be expected, but to find such a variation making a pattern in the form of a continuous strip through a field is decidedly out of the ordinary. The strip across the fields was marked by different species of plants from those on either side, by stunted growth of plants, or in some places, by their complete absence. The soil in this narrow area was observed to be lighter in color and drier than that on either side.

[2] The complete list of plants collected at Angel Mounds site will be published at a later date in connection with the archaeological report.

[3] A more complete discussion of the uses of native plants based on ethnobotanical literature will be published at a later date in connection with the archaeological report.

When viewed from the air, the difference between the plants forming the strip and those in the field on each side of it is far more striking, for the continuity of the whole can be seen to better advantage. The pattern extends through two wooded sections, and here, where cultivation did not interfere, a difference in elevation ranging from one to three feet is noticeable, the strip being more elevated. In the fields, variation in elevation is so slight that it is not easily discernible, but close contour surveying reveals a difference of a foot or slightly less still existing in most places.

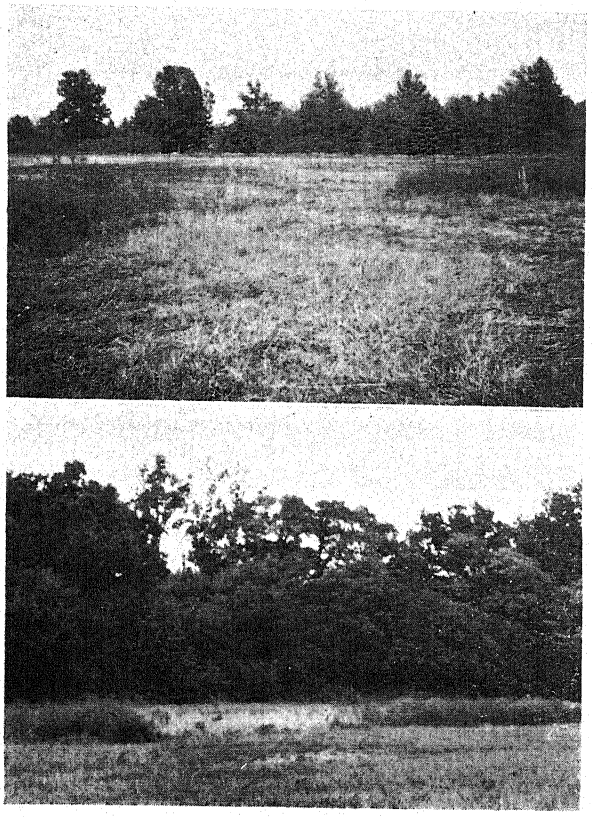


Fig. 2. Above, view of Transect I, looking from south to north across the transect, showing the site of the old wall crossing transect and field. The wall site is the lighter colored area marked by a distinct difference in plant growth. The tall growth at each side of the wall site is *Aster pilosus*. Below, view of Transect IV, looking from south to north across the transect, showing the site of the old wall crossing transect and field. The wall site is the lighter colored area marked by a distinct difference in plant growth. *Danthonia spicata* is growing on the wall site, with *Aster pilosus* on either side.

The farmers in the region attempted to explain the phenomenon by calling this an Indian road, but this was not a satisfactory hypothesis because a trail should be lower than the surrounding area, not higher; it went nowhere in particular, only leading in a somewhat circular fashion from the river around the mounds back to the river; and it was marked by distinctly wider areas at intervals of approximately 120 feet.

The archaeologists' early hypothesis was that this marked the location of a wall, surrounding an Indian village on the three sides not protected by the river. Excavation verified this hypothesis, and showed the wall to have been made of a single row of large logs set upright in a shallow trench, with soil heaped up around the base of the logs to help support them. Mr. Black believes that the wall surface was treated in the same manner as the walls of houses, with cane tied over the logs and the cane surface plastered with mud and straw. At intervals of approximately 120 feet along the wall, projecting bastions were found, explaining the wider areas previously mentioned. These provided a reasonably adequate means of protection when each was manned by an Indian with bow and arrow or equipped with a good supply of stones.

Method of study.—In an effort to discover an explanation for the marked plant differences along the old wall, six locations were selected at random, and a transect ten feet wide and approximately 100 feet long was staked off across the wall site at each location. Each transect was staked at ten-foot intervals throughout its length, so that it could easily be studied as a series of ten-foot squares. In addition, two transects which did not extend across the wall site were staked off in a similar manner. All transects were left unmowed, but the surrounding fields were mowed. The study of these transects, as well as of the unstaked areas, was begun in the spring of 1941 and carried through the autumn of 1942.

A careful record of floral distribution within the transects was kept, and the species and relative abundance of plants were recorded. String counts were tried, but they involved an immense amount of labor and gave the same graphic results as charting, so they were abandoned in favor of the latter method. The acidity of the soil, elevation, and soil nutrients were studied in relation to the plant distribution.

Readings on soil pH were made with a Beckmann glass-electrode meter. The first soil samples were taken at random across the transects at approximately three-foot intervals. Later samplings were taken every five feet through the center of the transects. The curves of the three-foot and five-foot samples were so similar that it was decided to use the five-foot samples. Samples were taken at depths of three inches and twelve inches, and at different times of the year. The soil was collected in half-pint waxed paper boxes, and readings were taken as soon as possible upon return to the laboratory. A series of samples read while fresh and again after drying for a month gave results so much alike that there seemed to be no point in making the determinations quickly, provided that all from one series were made at the same time.

A series of samples tested at Purdue University, by T. M. Bushnell, for potassium, phosphorus, and nitrates, did not show sufficient variation to warrant further study.

A section across the wall site parallel to a test transect was limed and observed during the second season.

Mr. Glenn Black and his staff were kind enough to take elevation readings corresponding with soil samplings, and, at the end of the study, to excavate sections of two of the test areas.

Results.—Results for each experimental area are presented separately for the sake of clarity.

Transect I. This was an area ten feet wide and 130 feet long, extending east and west across the field and wall site (fig. 1). Because of its convenient location and the conspicuous plant differences, the most extensive experimentation was carried out here.

Looking across the field at this transect, one could see a marked difference in the plants in the portion between 50 and 80 feet from the east end of the transect (fig. 2 and 3). Since this difference followed a pattern across the entire field, it was assumed that it indicated the position of the wall, and elevation readings gave credence to this idea. Although cultivation had leveled the field until there was a difference of only 1.1 feet between the highest and lowest elevation (377.2 feet and 376.1 feet above mean sea level) the highest parts occurred at exactly the place where the vegetation was different (fig. 2, 3, and 4). Soil sampling showed the acidity to be greatest in the same place, the pH falling from 5.00 and above to about 4.50 in this part of the transect (fig. 4). Tests for potassium, phosphorus, and nitrates showed no corresponding curves.

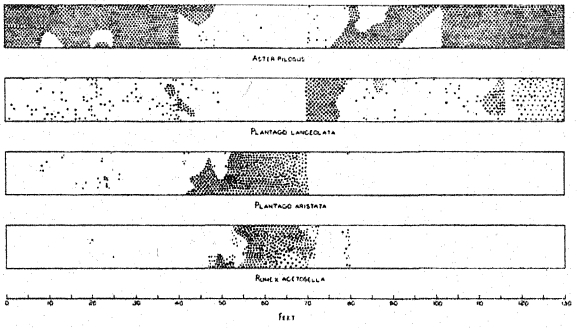


Fig. 3. Distribution of critical species within Transect I, 1941.

Floral distribution in this test area showed such little difference between the two years as to be negligible. The plants which appeared to be most sensitive to the difference in soil were *Aster pilosus, Plantago lanceolata, Plantago aristata,* and *Rumex acetosella.* Dense growth of *Aster* dropped out between 40 and 50 feet from the east end of the transect, and came in at about 80 feet, being absent on the presumptive wall site. *Plantago lanceolata* was lacking between 50 and 70 feet and scattered elsewhere, being absent on the wall site. *Plantago aristata* and *Rumex acetosella* occupied the more acid parts of the transect, being present on the wall site (fig. 2, 3). Apparently seeds of *Aster pilosus* and *Plantago lanceolata* which fall on the more acid part of the transect either do not germinate or die soon after, as seedlings were never seen here.

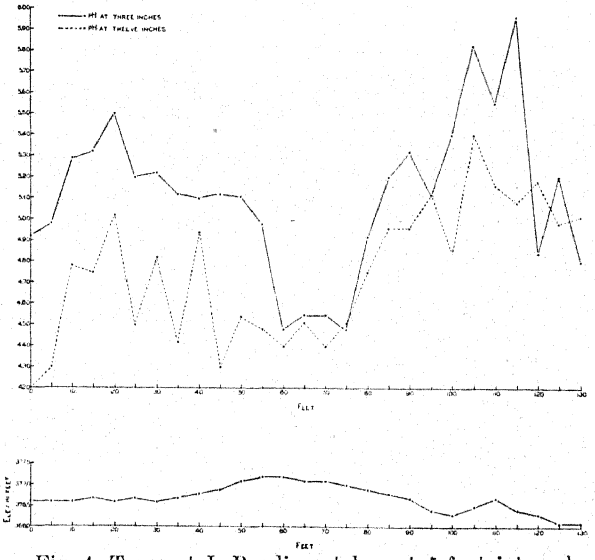


Fig. 4. Transect I. Readings taken at 5-foot intervals from east to west. Above, pH readings taken October 17, 1941; below, elevation readings.

The second season a transect parallel to Transect I and ten feet from it was limed with five pounds of air-slacked lime to each ten-foot block. This resulted in a much more luxuriant growth in the portion of the transect which crossed the site of the wall. This was particularly marked in the case of a few scattered plants of *Aster pilosus* growing on the wall site.

At the end of the second year, Mr. Black supervised the excavation of a trench five feet wide and 70 feet long beginning 30 feet from the east end of this transect and running westward to the 100-foot line. This disclosed that the trench which the Indians had dug into the subsoil for the reception of the palisade posts was located between 60 and 70 feet from the east end of the transect, and that soil samples removed at 65 feet were removed from the very center of the palisade trench. Since this was also the location of the greatest elevation, greatest acidity, and greatest plant differences, the excavations corroborated the belief that these conditions marked the site of the wall.

Transect II. This was a transect ten feet wide and 150 feet long, extending east and west across the field and wall site a short distance north of Transect I (fig. 1).

Elevation readings showed a variation of only

0.8 feet between the highest and lowest spots (377.2 and 376.4 feet). The highest elevations were in the portions between 50 and 85 feet, and between 120 and 145 feet from the east end of the transect. The wall site appeared to cross the transect between 50 and 85 feet.

The pH dropped from 5.00 and above to 4.39 at 75 feet from the east end of the transect. It also dropped at the other rise, but here the drop was not so great, being 4.90 at the 125-foot line.

Aster pilosus began dropping out at about 65 feet, and came in again at about 85 feet, being absent on the presumptive wall site. It occurred scattered between 120 and 145 feet. The rest of the transect was densely covered with this plant.

Between 70 and 80 feet *Rumex acetosella* and *Plantago aristata* occurred in abundance, but they were absent elsewhere. The growth of *Bromus commutatus* was dense everywhere except between the 65-foot and 75-foot lines, where the growth was light.

An excavation in this transect was begun, but weather conditions and shortage of labor prevented its completion. At some later date the results of this excavation will be reported by Mr. Black.

Transect III. This was a transect 110 feet long and ten feet wide, running east and west across a field which showed the pattern of the wall less clearly than any of the other fields which it crossed (fig. 1). The trend of the wall here was marked more by a variation in size and vigor of growth than by a difference of species.

Transect III was on a slight slope, making it somewhat difficult to correlate elevation with position of the wall without actual excavation.

The pH readings dropped between 40 and 50 feet from the east end of the transect, but the lowest readings were only 4.99 and 5.00. This was considerably higher than in Transects I and II, where the plant differences were striking. In Transect III *Aster pilosus* did not drop out, but only became smaller and less healthy in appearance. This plant covered the entire area, but was noticeably shorter between 40 and 50 feet than it was on either side of this area. *Erigeron annuus* dropped out between 35 and 60 feet, to be replaced by *Plantago aristata,* but the plantain here was less abundant and therefore less striking than in Transects I and II.

Transect IV. This transect, ten feet wide and 100 feet long, ran east and west through a third field (fig. 1).

Again, the elevation differences within the transect were slight, only 0.8 foot, ranging from 378.5 feet to 377.7 feet. The greatest elevation occurred within the part 45 to 75 feet from the east end of the transect. The greatest acidity was also between 45 and 80 feet, the pH dropping to about 4.60 here (fig. 5). The plant difference was also greatest between 45 and 85 feet. *Aster pilosus* began to drop out abruptly at about the 50-foot point, and was gone entirely at 60 feet. It began to reappear at about 90 feet, and, at approximately 95 feet, dense growth was resumed abruptly. *Danthonia spicata* occupied the more acid space where the *Aster* was absent. *Panicum huachucae* grew in the same area as *Aster,* dropping out to make way for the *Danthonia* (fig. 2, 5).

Transect V. This transect was 70 feet long by ten feet wide, running north and south through a dry woods (fig. 1).

The slight rise left by the remains of the old wall could be seen here, although there was only a one-foot difference between the highest and lowest elevation readings (378.2 and 377.2 feet). The greatest height was between 20 and 30 feet from the south end of the transect. The elevation began to rise with the ten-foot reading, and dropped between 30 and 40 feet.

The first half of the transect, up to the 35-foot line, is more acid than the latter half, with readings of about 5.00 in the most elevated portion.

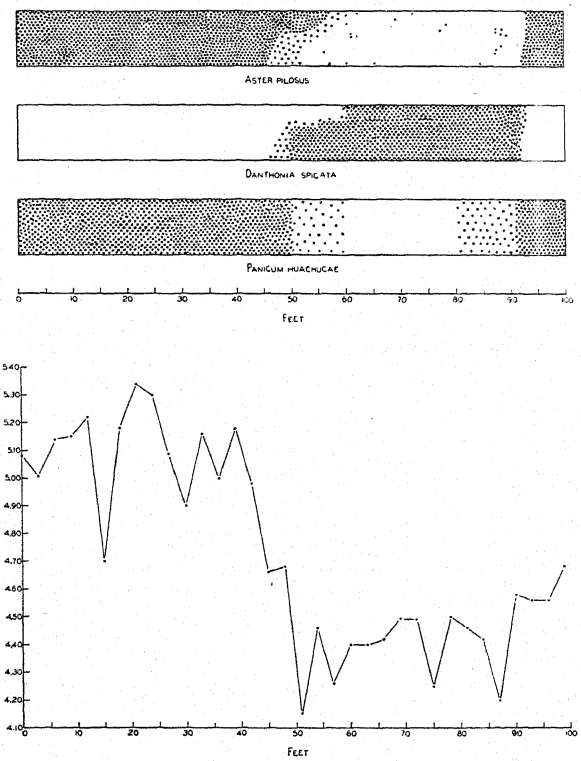


Fig. 5. Transect IV. Above, floral distribution of critical species, 1941. Below, pH readings taken at a depth of 3 inches at approximately 3-foot intervals from east to west across the transect, September 1, 1941.

Danthonia spicata was found between ten and 35 feet from the south end of the transect. No other species was limited in this way, but all present were scattered over the area. With the exception of *Danthonia,* none of the species which were critical in the field areas grew here.

Transect VI. Transect VI, ten feet wide and 130

feet long, ran north and south across both woods and field (fig. 1). The wooded portion sloped down into a swampy area which was covered with water in the spring and early summer. The remains of the wall were most prominent in the woods. The greatest elevation was found to be between 55 and 85 feet from the south end of the transect, with a difference of 7.75 feet between the highest (381.0 feet) and lowest (373.25 feet) elevations for the entire transect. The north end of the transect slopes down into the swampy woods, and this accounts for the great difference in elevation, but the rise of the wall from the south or field side is very marked and represents a difference of about three feet from the field level.

The greatest acidity (pH 4.20) was found in the highest part of the transect, between 55 and 80 feet from the south end.

During the first year, the transect was altogether bare of plants between 65 and 70 feet, with a very sparse growth between 50 and 75 feet. This was in part due to the fact that a foot path through the woods followed the higher ground along the wall. The second year the bareness was also prominent, but a few plants of *Danthonia spicata* were found here. *Aster pilosus* still grew in the field portion of the transect, but the dense growth ended abruptly near the 40-foot mark, and scattered plants occurred in the next ten feet. Its growth was resumed at about 75 feet, but in this wooded part of the transect the growth was never as heavy as in the field portion.

It was just east of Transect VI where the first excavation had been made which showed this definitely to be a part of the village wall.

Transects not extending across wall.—The first of these was laid out on the side of Mound A. Here the soil samples were all highly alkaline. There appeared to be no correlation between soil acidity, elevation, and floral distribution; but it was rather a question of drainage and the effects of previous cultivation.

The second area was laid out across what was believed to be an old game court in the middle of the Indian village. Here plants grew in general all over the entire transect except where they were killed by standing water in the spring and early summer. The lowest pH reading was 5.00 and those immediately preceding and following were 5.50. At this spot, *Aster pilosus* made a noticeable change from a dense to a scattered growth, but this spot also represented the beginning of the wet area, and in another 20 feet standing water prevented growth of any plants. It seems probable in this case that drainage was more important than acidity, although both were contributing factors.

Comparison of results.—Comparing the results for all the transects, it is apparent that there is a definite correlation between pH, elevation, and floral distribution of certain "indicator" species. When the pH falls to 5.00 and below, *Aster pilosus* is stunted in growth or absent, depending upon the degree of acidity. *Plantago lanceolata* is also intolerant of the more acid regions. *Plantago aristata, Rumex acetosella,* and *Danthonia spicata,* on the other hand, grow well with the pH below 5.00, and are not tolerant of less acid soils. Where the pH difference is greatest, the plant difference is most striking. This may readily be seen by comparing Transects I and III.

Elevation readings, though the differences may be very slight, are correlated with the distributions of critical species and with soil acidity, and all this is definitely superimposed upon the position of the village wall as shown by excavation.

Discussion.—From a study of these results, it seems apparent that certain common plants growing in the region are sensitive to pH differences of slight degree. This in itself is not to be wondered at, for it is well known that plants have different affinities in this respect. What is unusual is that the acid parts of the field extend in a line which can readily be traced because of plant differences and which also corresponds to a slight rise in elevation. This brings up the question as to whether an abnormal plant growth of this sort might indicate to archaeologists a possible place to excavate. Excavations made across the wall at Angel site would seem to support this idea, since they showed the wall to be where it was predicted on the basis of such plant differences.

Although the study strongly indicates that the peculiar plant distributions were due to acidity differences, continued research may show that some as yet undetermined physical characteristic of the soil has greater effect on the plant growth than the pH itself.

While three important plant nutrients appeared not to be correlated with other variables, it is possible that the situation was complicated by toxic minerals. The increased vigor of plants on the limed area suggests that if such substances were present, they were not as important as acidity in affecting the growth of the plants.

In parts of the wall used in recent years as footpaths, walking may have packed and altered the soil. However, in no case was a texture difference noted between the soil on the wall site and that on either side.

The soil in bare or very sparsely populated parts of transects was observed to be very dry. This would seem, however, to be due to the absence of plants rather than to texture differences (all a fine clay silt) which would affect the water-retaining powers. This conclusion is drawn after comparing bare parts of the wall site with artificially denuded areas off the wall which become extremely dry upon removal of plants. If the Indians at the time of occupation were in the habit of walking next to the wall, such trampling might have compacted the soil, and such compaction might still persist and be a contributing factor to the dryness of the wall site.

However, there is no evidence that there were such paths.

The growth of plants on other denuded areas, such as those created in excavating, or in paths and road tracks, emphasizes the peculiarity of the growth on and along the site of the wall. In these other areas, plants similar to the surrounding growth quickly come in, or rank weeds common to the region on newly cultivated ground make their appearance. If the plant differences on the wall site were due to soil moisture alone, we should get the same effect on the wall as on these other bare areas. Accelerated plant growth after liming also points away from soil moisture and towards acidity as the cause of plant differences. Also seedlings of acid-intolerant plants do not appear on the wall site even when the soil is moist in the spring. It seems obvious that the wall must have been responsible for creating soil differences which exist to this day. Just how this was accomplished is open to conjecture.

There is nothing to support the view that the soil piled up around the base of the logs to hold them in position was brought in from some other place, and it seems highly improbable that this would have been done when one considers how much easier it would have been to use the soil at hand. The inherent differences are no doubt due to other causes.

There is no doubt that we are faced with a problem of peculiar deposition. Over a period of years soil was washed down from the mud-plastered wall by rains. It is probable that there might have been a continuous deposit of one hundred years or so of clay with the soluble lime salts washed out of it, resulting in acidity due to a deficiency of soil bases or absence of reserve calcium carbonate (Russell, 1927; Waksman, 1932).

Another point to be considered is the large logs which made the backbone of the wall. As the logs decayed, together with the mud-covered cane and grasses with which they were plastered, organic acids were freed. If the soil which was piled up around the logs had had the calcium bases leached out over a long period of years, there would not have been enough left to counteract the acids so formed and they might have persisted.

At present there seems to be no way to determine which of these possible explanations, if any, is the correct one, or whether they were all contributing factors. They are set forth as thought-provoking possibilities, and are not intended as conclusive evidence of what may have happened.

SUMMARY

A botanical investigation made at Angel Mounds site during the growing seasons of 1941 and 1942 consisted of two parts: a survey of the vascular plants of the region, and a study of plant differences on and along the old village wall.

During the survey, 339 different species of vascular plants were collected and identified, and six additional species were identified to the genus only. Of the 339 species, 241 were new records for Vanderburgh County. Several of the species found have rarely been reported from Indiana.

The study of the site of the old village wall showed a definite correlation between pH, elevation, and the distribution of certain "indicator" plants. In general, where the elevation is greatest, that is, where the stockade originally stood, the pH is lowest. There is a definite tendency for certain plants such as *Aster pilosus* Willd. and *Plantago lanceolata* L. to grow up to the point where the elevation rises and the pH drops, then thin out abruptly to the point of disappearance, while other plants such as *Plantago aristata* Michx. or *Danthonia spicata* (L.) Beauv. grow only on the more acid part of transects, thinning out altogether where the elevation drops and the pH rises. In transects where the pH differences are not marked, the plant differences are also much less marked. Excavation supported these observations, and indicated that soil acidity and plant distribution provided a method of predicting the position of the wall before excavations were made.

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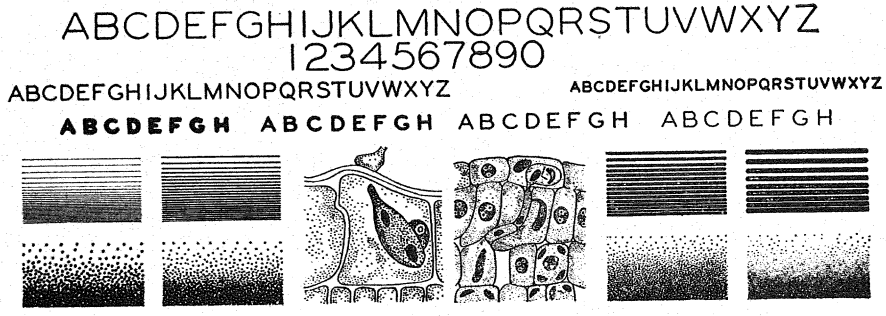
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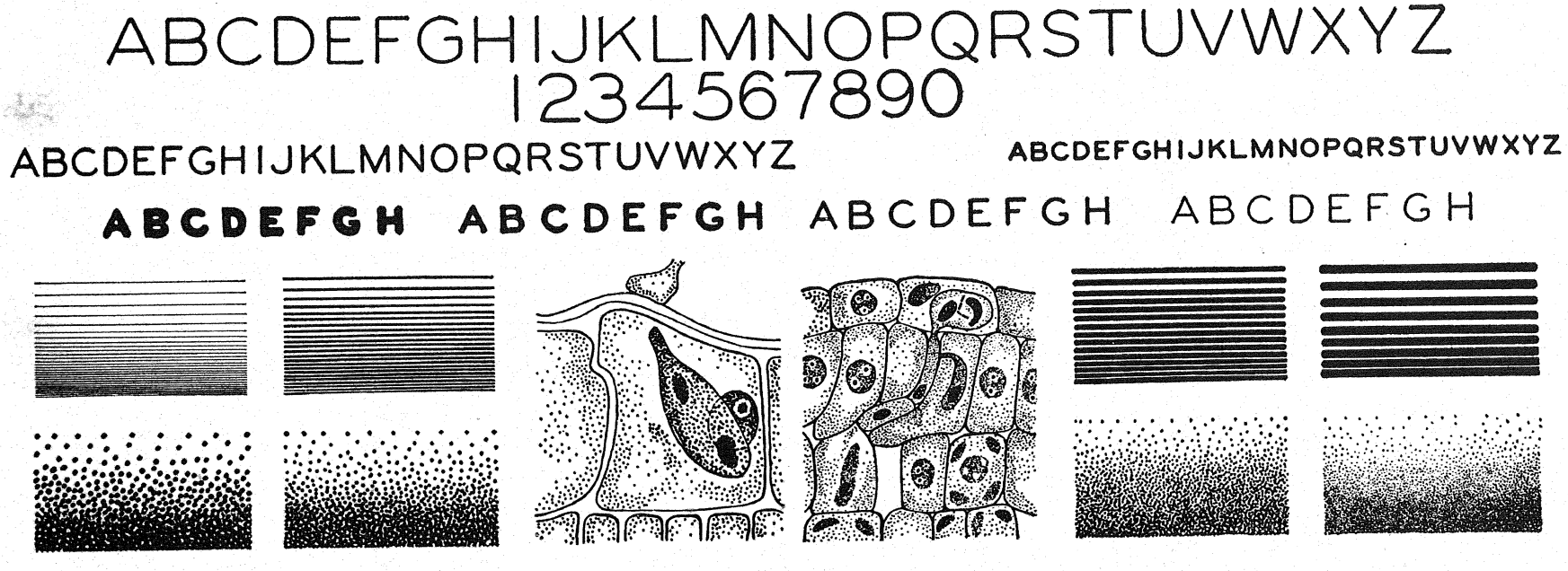
DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to ¼. Middle—Reduction to ½. Bottom—Original size.

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Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.



STUDIES OF A GENETIC DISEASE OF TRIFOLIUM REPENS SIMULATING A VIROSIS [1]

S. S. Atwood[2] and K. W. Kreitlow[3]

SEVERAL STUDIES have been made of plant diseases where genetic factors cause deformities similar to those caused by pathogenic agents. A correct diagnosis of such a condition occasionally proves difficult, particularly if the principal symptoms consist of irregular mottling of the leaves, but such cases are of especial interest to biologists, since they provide good examples of the fact that either environmental or heritable factors may induce a similar type of morphological response.

One of the first cases of a heritable leaf blotch was reported by Emerson (1923) in corn, where a single recessive factor is principally responsible for considerable areas of the leaves turning yellow and dying. Many variations in the degree of blotching were observed, caused in part by the action of modifying factors, but in general the symptoms resembled those associated with particular leaf-spot diseases of certain fruit trees. A similar condition was described by Ferdinandsen and Winge (1930) in oats, but in this case the inheritance was attributed to cytoplasmic rather than chromosomal factors. More recently a well defined case was described in *Datura*. Blakeslee (1921) found first a type called "quercina," which subsequently was ascribed to a virus disease. Later Blakeslee and Avery (1941) reported a recessive gene mutation, which was induced through X-ray treatment of seeds. Both the virus and the mutant gene caused elimination of spines from the capsule, separation of the tubular corolla into individual petals, extension of the stigmatic surface on the pistils, and production of narrow, eroded leaves.

Several authors have reported abnormalities resembling symptoms of virus infection in the common bean *Phaseolus vulgaris*. Burkholder and Muller (1926) described a recessive hereditary abnormality which they called "pseudo-mosaic" because of its close resemblance to true mosaic of bean. Parker (1934), studying a leaf variegation of beans that was not transmitted artificially, concluded that the inheritance of the abnormality was governed by the cytoplasm or its inclusions. Zaumyer (1938) investigated a leaf variegation resembling mosaic in hybrid beans. The symptoms appeared when the variety Corbett Refugee was used either as male or female parent.

The case reported here for white clover is similar to those in *Datura* and *Phaseolus* since the observed injury resembles the mottling or mosaic caused by a virus infection.

MATERIALS AND METHODS.—The female parent of the plants used in this investigation was selected because of the severe mottling or mosaic of its leaves. When this plant was first noticed in the field in 1940, it appeared to be tolerant to the injury, and it continued to grow vigorously despite the abundance of symptoms, whereas most virus-infected plants of clover in the same field were severely stunted. For this reason it was thought that this individual might provide germplasm for a study of the genetics of susceptibility to virus infection.

The male parent was unrelated to the female and was free of mottling. All progeny plants used in the genetic investigations descended from the cross between these two parents. The controlled crosses to produce the various progenies were made during the winter in screened greenhouses, following the procedures found useful in studies of incompatibility (Atwood, 1941). The classification of advanced generation plants was made on seedlings grown individually in 4-inch pots in the greenhouse. In the inoculation and grafting experiments, various methods were used; these will be noted in detail in connection with the descriptions of these studies.

The values of chi-square were corrected for continuity wherever necessary according to the suggestions of Cochran (1942).

EXPERIMENTAL RESULTS.—The cross between the parent plants was made in the winter of 1941–42, and the F_1 progeny was space-planted in the field the following summer. The 45 F_1 plants segregated clearly, 12 plants showing severe injury like their mottled parent, and 33 showing no symptoms. The symptoms of mottling became evident very early in the development of the plants, and they persisted throughout the life of the plants during two summers in the field. All mottled plants were less vigorous than the healthy, and most of them died back severely during the winter. In their second summer the combined virus-like injury and winterkilling so weakened the mottled plants that they were easily distinguished from the healthy on the basis of vigor alone (fig. 1).

Because the mottled F_1 plants showed symptoms when grown in the field without inoculation and because no change in classification was noted throughout the two years in the field, it was postulated that the mottled plants were not injured from virus in-

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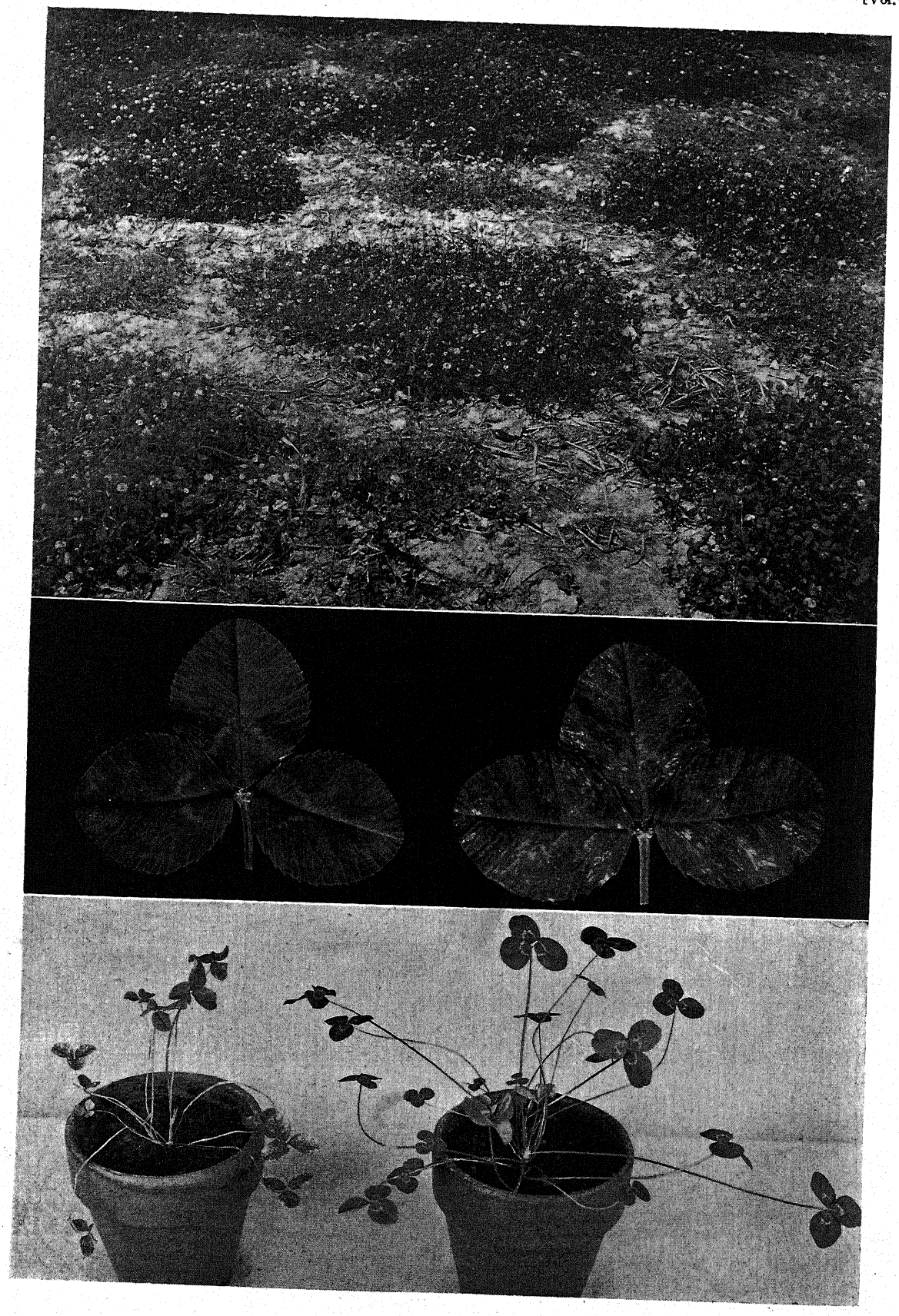


TABLE 1. *Postulated genotypes for parents and F_1 plants, together with expected segregations in B and F_2 families.*

Parental genotypes	Ratio of abnormal:healthy expected in B families	F_1 genotypes	Ratio of abnormal:healthy expected in F_2 families
Mottled ♀ AaBb	9:7	1 mottled AaBb	9:7
	3:5	3 healthy {Aabb} {aaBb}	3:5
	1:3		1:3
Healthy ♂ aabb	1:3		1:3
	no seg.		
	no seg.	aabb	no seg.

fection but rather as a result of some heritable abnormality. The 12:33 segregation obtained with the F_1 plants fits a 1:3 ratio closely (corrected $\chi^2 = 0.022$) and may be explained by the assumption of two complementary dominant factors determining the mottled condition, the female parent being a double heterozygote and the male homozygous recessive. Subsequent studies were planned to test this hypothesis and to ascertain, if possible, the cause of the mottled condition.

Backcross progenies.—In the winter 1942–43 eleven selected F_1 plants (five mottled and six healthy) were intercrossed and backcrossed to both parents. The progenies were grown in the greenhouse and classified in the seedling stage. The seeds were germinated in peat moss, and all resultant seedlings were transplanted individually to soil in clay pots about the time the seedlings had developed one or two trifoliolate leaves.

The first symptoms of injury were evident on some seedlings before transplanting, but they became more distinct after three or four trifoliolate leaves had developed. By the time the plants were six weeks old, segregation was very well defined; there were no cases of slight or intermediate injury. Each plant was noted again at the age of two months, and in no case did the new classification differ from the earlier one. Most of the 2,278 F_2 and backcross plants were discarded at this stage, but over 100 were grown to maturity for further studies of their genetic type, inoculation methods, and growth following grafting. None of these plants showed a change of type, and none of them lost their symptoms, as is sometimes the case with virus infected individuals.

According to the complementary dominant factor hypothesis used to explain the F_1 segregation, four different ratios (table 1) should be obtained in the backcrosses (B). The cross mottled P × mottled F_1 (and reciprocal) was expected to yield a 9:7 ratio of mottled to healthy plants, but only three (Nos. 2, 3, and 4) of the five families (Nos. 1 to 5, table 2) fitted this expected ratio satisfactorily, based on the corrected value of χ^2. In each of the five families, there was a deficiency of mottled plants, and this consistent trend made both total χ^2 and χ_t^2 significant. From the cross mottled P × healthy F_1 (and reciprocal), on the other hand, two types of segregating ratios were expected, depending on the genotype of the F_1 plant. With two (Nos. 9 and 10) of the six resultant families (Nos. 6 to 11, table 2) the obtained ratios fitted a 1:3 satisfactorily but deviated significantly from a 3:5; three other families (Nos. 6, 8, and 11) gave ratios fitting both expected types, while the fourth (No. 7) deviated significantly from both ratios. Evidence from the F_2 data, however, indicated that two of the healthy F_1 plants must have been heterozygous for one factor while four were homozygous recessive for both. When the six backcross families were grouped accordingly, the individual χ^2 values as well as the totals showed a satisfactory fit to expected with the four double homozygotes, but with the other two F_1 plants, the χ^2 for one family as well as total χ^2, χ_t^2 and χ_h^2 were all highly significant.

Four of the five mottled F_1 plants were backcrossed to the healthy parent, and all resultant families (Nos. 12 to 15, table 2) gave segregations that fitted satisfactorily the expected 1:3 ratio. Likewise when the six healthy F_1 plants were backcrossed to

Fig. 1–3.—Fig. 1 (above). Part of the F_1 population in the field, photographed near the end of the flowering period during the second summer. All small plants have the abnormality, while the large plants are healthy.—Fig. 2 (center). On the left is a normal leaf of white clover, and on the right is a leaf with early symptoms of mottling.—Fig. 3 (below). On the right is a normal seedling and on the left an abnormal one from the same segregating family, photographed when the seedlings were six weeks old. The necrotic areas on the blades, the smaller number and size of leaves, and the twisted petioles and blades were characteristic of the abnormal plants.

TABLE 2. *Segregations for abnormal and healthy plants in backcross populations.*

Origin and expected ratio	Family number	Cross	Number of plants mottled	healthy	total	Corrected χ^{2a} for expected ratio	
Mottled ♀P	1	♀P × 1 & recip.	30	49	79	— 9.992[b]	
×	2	♀P × 2 & recip.	55	62	117	— 3.694	
mottled F_1	3	♀P × 3	16	16	32	— 0.572	
(and recip.)	4	♀P × 4 & recip.	50	49	99	— 1.105	
9:7	5	♀P × 5 & recip.	14	33	47	—12.320[b]	
		Total	165	209	374	30.500[b]	
					χ_t^2	—21.879[b]	
					χ_h^2	8.130	
Mottled ♀P	6	♀P × 9 & recip.	33	64	97	— 0.364	+ 3.742
×	7	♀P × 10 & recip.	53	29	82	+24.615[b]	+66.602[b]
healthy F_1		Total	86	93	179	26.260[b]	72.909[b]
(and recip.)		(heterozy. for 1 factor)			χ_t^2	+ 8.048[b]	+49.477[b]
3:5					χ_h^2	17.768[b]	22.211[b]
or							
1:3	8	♀P × 6 & recip.	22	51	73	— 1.389	+ 0.772
	9	♀P × 7 & recip.	11	56	67	—11.822[b]	— 2.194
	10	♀P × 8 & recip.	22	67	89	— 5.670[c]	— 0.034
	11	♀P × 11 & recip.	15	35	50	— 0.901	+ 0.427
		Total	70	209	279	21.797[b]	4.330
		(homo. recess. for both)			χ_t^2	—17.809[b]	+ 0.001
					χ_h^2	3.463	4.329
Healthy ♂P	12	♂P × 1 & recip.	20	36	56	+ 2.881	
×	13	♂P × 2 & recip.	32	91	123	+ 0.024	
mottled F_1	14	♂P × 3 & recip.	19	42	61	+ 0.923	
(and recip.)	15	♂P × 5 & recip.	15	45	60	○ 0.222	
1:3		Total	86	214	300	4.727	
					χ_t^2	+ 1.960	
					χ_h^2	2.576	
Healthy ♂P	16	6 × ♂P	0	33	33	.....	
×	17	7 × ♂P	0	37	37	.....	
healthy F_1	18	8 × ♂P	0	41	41	.....	
(and recip.)	19	9 × ♂P	0	23	23	.....	
No segregation	20	10 × ♂P	0	20	20	.....	
	21	11 × ♂P & recip.	0	57	57	.....	
		Total	0	211	211	.....	

[a] The + sign before the χ^2 value indicates an excess of mottled plants; the — sign indicates a deficiency; and ○ indicates an exact fit to expected.
[b] Highly significant—exceeds χ^2 for P = .01.
[c] Significant—exceeds χ^2 for P = .05.

their healthy parent, all of the 211 resultant progeny (families 16 to 21, table 2) were healthy as expected.

F_2 progenies.—When the same hypothesis (table 1) was applied to the F_2 data (table 3), a good fit to expected was obtained in most of the families. The cross mottled × mottled was expected to yield a 9:7 segregation of mottled to healthy, and this was obtained in two (Nos. 22 and 23) of the three families (Nos. 22 to 24, table 3). Here as with the backcrosses segregating 9:7 there was a deficiency of mottled plants in every case, and in one family (No. 24), the deviation was so large that both total χ^2 and χ_t^2 were highly significant.

When mottled F_1 plants were crossed with healthy, two types of segregations were expected, depending on the genotype of the healthy plants involved. On the basis of these and other results, two healthy plants were considered to be heterozygous for one of the factors. In two (Nos. 26 and 28) of the six families (Nos. 25 to 30, table 3) from crosses involving these two plants, the ratios fitted 3:5 but deviated significantly from 1:3. In two other families (Nos. 29 and 30) the reverse was true, while in the remaining two (Nos. 25 and 27), the obtained segregations fitted both expected ratios. In these families, as in those segregating 9:7, there was a deficiency of mottled plants, particularly in the two families that deviated significantly from 3:5. On the other hand, with the four plants assumed to be homozygous for both factors, five (Nos. 31, 33, 36, 38, and 41) of the 11 families (No. 31 to 41, table 3) fitted 1:3 but deviated significantly from 3:5. One other (No. 37) differed significantly from both 1:3 and 3:5, but the deviation from 3:5 was greater than 1:3. The other five families gave satisfactory fits to both expected ratios. Among these eleven families,

TABLE 3. *Segregations for abnormal and healthy plants in F_2 populations.*

Origin and expected ratio	Family number	Cross	Number of plants mottled	healthy	total	Corrected χ^2[a] for expected ratio	
Mottled	22	1 × 2 & recip.	31	36	67	— 2.322	
×	23	1 × 3	12	12	24	— 0.174	
mottled	24	2 × 4	6	23	29	—13.492[b]	
9:7		Total	49	71	120	18.048[b]	
					χ_t^2	—10.971[b]	
					χ_h^2	6.459	
Mottled	25	9 × 2	2	15	17	— 3.769	— 0.960
×	26	9 × 3	5	3	8	+ 1.200	+ 4.167[c]
healthy	27	10 × 1 & recip.	18	41	59	— 0.950	— 0.684
(and recip.)	28	10 × 2	17	18	35	+ 2.333	+ 9.152[b]
3:5	29	10 × 3	3	22	25	— 5.891[c]	— 1.613
or	30	10 × 5	3	19	22	— 4.376[c]	— 0.970
1:3		Total	48	118	166	23.345[b]	22.677[b]
		(heterozy. for 1 factor)			χ_t^2	— 4.859[c]	1.157
					χ_h^2	18.126[b]	21.320[b]
	31	6 × 4	8	34	42	— 5.340[c]	— 0.508
	32	6 × 5	10	31	41	— 2.473	— 0.081
	33	7 × 1 & recip.	14	49	63	— 5.639[c]	— 0.259
	34	7 × 2	12	20	32	○ 0.021	+ 2.042
	35	7 × 4	7	21	28	— 1.372	○ 0.008
	36	8 × 1	4	27	31	—12.751[b]	— 1.817
	37	8 × 3	2	34	36	—17.785[b]	— 6.259[c]
	38	8 × 5	2	19	21	— 5.870[c]	— 1.921
	39	11 × 1 & recip.	16	45	61	— 2.843	+ 0.005
	40	11 × 2 & recip.	8	23	31	— 1.344	+ 0.011
	41	11 × 4	2	19	21	— 6.488[c]	— 1.921
		Total	85	322	407	70.506[b]	18.778
		(homo. recess. for both)			χ_t^2	—47.235[b]	— 3.460
					χ_h^2	22.565[c]	15.102
Healthy	42	8 × 9 & recip.	0	45	45	.....	
×	43	8 × 11 & recip.	0	39	39	.....	
healthy	44	9 × 10 & recip.	16	39	55	+ 0.297	
1:3	45	9 × 11 & recip.	0	39	39	.....	
or	46	10 × 6	0	22	22	.....	
no segregation	47	10 × 7	0	20	20	.....	
	48	10 × 8	0	22	22	.....	
		Total (not segregating)	0	187	187	.....	

[a] The + sign before the χ^2 value indicates an excess of mottled plants; the — sign indicates a deficiency; and ○ indicates an exact fit to expected.
[b] Highly significant—exceeds χ^2 for P = .01.
[c] Significant—exceeds χ^2 for P = .05.

there was a trend toward deficiency of mottled plants when fitted to a 1:3 ratio, but none of the deviations were excessive and total χ^2, χ_t^2, χ_h^2 all gave satisfactory values.

From the cross healthy × healthy, a 1:3 segregation was expected if the two plants involved were heterozygous for different factors; any other combination should produce only healthy progeny. One family (No. 44, table 3) segregated 16:39, which fits the expected 1:3 satisfactorily. The other six families in this class (Nos. 42 to 48, table 3) produced only healthy plants among the 187 progeny examined.

On the basis of the B and F_2 results, it appeared that one of the six healthy F_1 plants was heterozygous for one factor, one plant was heterozygous for the other factor, while four plants were homozygous recessive for both. This 1:1:4 segregation fits the expected 1:1:1 with $\chi^2 = 3.000$.

Progeny tests of F_2 and B.—Since there were many inconclusive features in both the F_2 and B results, selected plants from these families were backcrossed to both original parents for a progeny test. Three groups of plants were used. The first group consisted of 11 mottled plants (Nos. 12 to 22, table 4) selected from F_2 families segregating 9:7. According to the hypothesis these could include three types, which would yield the ratios shown in table 6 when backcrossed to their original parents.

Of the seven families obtained from backcrossing to the mottled parent, four (Nos. 12, 17, 19 and 20) gave a satisfactory fit to 9:7 but deviated signifi-

TABLE 4. *Segregations for abnormal and healthy plants in progeny tests of selected F_2 and backcross individuals.*

Origin of the selected F_2 and backcross plants used in tests of their backcross progeny	F_2 or backcross plant no.	Backcrosses with mottled ♀P: Number of plants mottled	healthy	total	Corrected χ^{2a} for indicated ratios 9:7	3:1	Backcrosses with healthy ♂P: Number of plants mottled	healthy	total	Corrected χ^{2a} for indicated ratios 1:3	1:1
Mottled plants from F_2 families, Nos. 22 and 24, segregating 9:7	12	35	38	73	— 1.722	—27.073[b]	16	57	73	— 0.224	—21.918[b]
	13	..	..	..	.....	.....	18	40	58	+ 0.828	— 7.603[b]
	14	28	45	73	— 8.785[b]	—50.342[b]	10	38	48	— 0.250	—15.188[b]
	15	16	25	41	— 4.268[c]	—26.415[b]	21	68	89	— 0.034	—23.775[b]
	16	..	..	..	.....	.....	0	13	13	— 3.102	—11.077[b]
	17	20	20	40	— 0.406	—12.033[b]	14	13	27	+ 9.000[b]	+ 0.000
	18	22	48	70	—16.531[b]	—68.571[b]	12	63	75	— 2.778	—33.333[b]
	19	11	10	21	— 0.019	— 4.587[c]	..	..	..	.....	.....
	20	3	7	10	— 1.835	— 8.533[b]	6	40	46	— 2.899	—23.674[b]
	21	..	..	..	.....	.....	16	28	44	— 2.455	— 2.750
	Total	135	193	328	37.575[b]	209.817[b]	113	360	473	26.527[b]	148.645[b]
				χ_t^2	—29.745[b]	—198.541[b]	..	..	..	— 0.254	—127.941[b]
				χ_h^2	7.220	9.476	..	..	..	26.216[b]	19.662[c]
	22	..	..	..	.....	.....	30	34	64	+15.188[b]	— 0.141
					9:7					1:3	
Mottled plants from B family, No. 15, segregating 1:3	23	26	21	47	— 0.000		6	13	19	+ 0.158	
	24	22	17	39	+ 0.020		14	54	68	— 0.490	
	25	9	13	22	— .527		16	50	66	— 0.000	
	26	14	26	40	— (502[c]		17	60	77	— 0.212	
	27	17	19	36	— 0.854		18	45	63	+ 0.259	
	28	..	..	..	... ...		23	48	71	+ 1.695	
		88	96	184	1(653		94	270	364	4.015	
				χ_t^2	— 4 969[c]		..	..	..	+ 0.092	
				χ_h^2	5 347		..	..	..	3.883	
					3:5	1:3				all healthy	
Healthy plants from B family No. 15, segregating 1:3	29	..	..	..	.....	.....	0	44	44	.....	
	30	..	..	..	.....	.....	(1)	39	40	.....	
	31	..	..	..	.....	.....	0	13	13	.....	
	32	9	21	30	— 0.436	+ 0.178	1	35	36	.....	
	33	..	..	..	.....	.....	2	38	40	.....	
	34	10	24	34	— 0.635	+ 0.157	0	1	1	.....	
	35	10	29	39	— 1.862	+ 0.009	..	..	..	.....	
	36	5	14	19	— 0.593	+ 0.018	..	..	..	.....	
	37	8	29	37	— 3.332	— 0.081	0	38	38	.....	
	38	6	29	35	— 5.350[c]	— 0.771	..	..	..	.....	
		48	146	194	15.192[c]	2.157	4	208	212	.....	
		..	..	..	—12.933[b]	+ 0.000	..	..	..	.....	
		..	..	..	1.720	2.150	..	..	..	.....	
	39	16	15	31	+ 2.067	+10.333[b]	1	25	26	.....	
	40	..	..	..	.....	.....	6	33	39	.....	

[a] The + sign before the χ^2 value indicates an excess of mottled plants; the — sign indicates a deficiency; and ○ indicates an exact fit to expected.
[b] Highly significant—exceeds χ^2 for P = .01.
[c] Significant—exceeds χ^2 for P = .05.

cantly from 3:1. The other three (Nos. 14, 15 and 18) gave ratios significantly different from both expectations, but in every case the fit to 9:7 was closer than to 3:1. Here as in the B and F_2 results there was a consistent deficiency of mottled plants, and both total χ^2 and χ_t^2 for 9:7 were highly significant. Nine families were obtained by backcrossing to the healthy parent, and seven (Nos. 12, 13, 14, 15, 16, 18 and 20) of the resultant segregations fitted 1:3 while differing significantly from 1:1. One other family (No. 17) gave a segregation fitting 1:1 but not 1:3, and the one remaining family (No. 21) fitted both expected ratios. In the case of plant 17, the backcrosses to the mottled parent fitted 9:7 better than 3:1, but the backcrosses to the healthy parent fitted 1:1 better than 1:3. These results are contra-

TABLE 5. *Average number of dominant factors and the number of leaves for abnormal and healthy plants in the four types of segregation ratios.*

Expected segregation ratio	Mottled plants			Healthy plants		
	Ave. no. dominant factors	Average number leaves	Range in family averages	Ave. no. dominant factors	Average number leaves	Range in family averages
9:7	2.67	4.41	3.28–5.62	1.14	6.45	4.12– 8.41
3:5	2.33	5.08	4.00–6.36	1.00	8.13	6.17–10.67
1:3	2.00	5.37	3.57–8.00	0.67	8.98	4.91–15.91
No segregation	...	...	.....	0–0.50	11.51	5.91–16.15
Least signif. diff. (1%)	...	0.69	.....	.....	1.64	.....

dictory, so for summary purposes plant 17 is included where the fit is better, namely, among the double heterozygotes. Likewise with plant 21, which gave a ratio that fitted both possible types; it is grouped with the class showing the lower χ^2.

When the eleven F_2 plants were summarized according to their progeny test, it was seen that none were homozygous dominant for both factors, only one appeared to be heterozygous for one factor, while the other ten appeared to be heterozygous for both. This ratio of 0:1:10 differs significantly from the expected ratio of 1:4:4. If one of the two questionable plants is classified into its less likely type, the resultant ratio of 0:2:9 still differs significantly from expectation ($\chi^2 = 6.384$). It is suggested that the plants tend to be lethal when one or both of the factors are homozygous dominant. This would account for the unexpected results in the progeny tests and would also explain the consistent deficiency of mottled plants in many of the families segregating 9:7 or 3:5.

When six mottled plants (Nos. 23 to 28, table 4) from a B family segregating 1:3 were backcrossed to both original parents, the obtained ratios fitted expected in every case except one. Here as with other families segregating 9:7 there was a large deficiency of mottled plants.

TABLE 6.

		Mottled AaBb P	Healthy aabb P
1	AABB	all mottled	all mottled
4	AaBB or AABb	3:1	1:1
4	AaBb	9:7	1:3

Healthy plants (Nos. 29 to 40, table 4) from the same B family likewise were progeny tested. Among the backcrosses to the mottled parent, one family (No. 39) fitted 3:5 but not 1:3, and one other (No. 38) fitted 1:3 but not 3:5. All others gave satisfactory fits to both possible ratios. Backcrosses of these plants to their healthy parent were expected to give only healthy offspring, but in five of the families mottled plants were noted. By mistake all except one of these plants were discarded, but with the one plant which was saved (noted in parentheses opposite F_2 plant No. 30, table 4) a later observation showed that it was small and feeble but healthy as far as the necrotic areas on the leaves were concerned. It is suggested that the four mottled plants noted in the families from plants 32, 33, and 39 similarly may have been misclassified. In the family from plant 40, which gave a ratio of 6:33, there is a possibility that the six mottled plants were misclassified, but there is also the possibility that a mistake was made in classifying plant 40 itself. If this is so, it appears to be the only case of this sort in the entire study.

Data on number of leaves and stand.—When the F_2 and B plants were being classified, it was noticed that healthy plants could often be distinguished from the mottled on the basis of vigor alone. To obtain a measure of this difference, the number of completely opened trifoliolate leaves was counted when the plants were six weeks old. For the entire F_2 and B population of 2,278 plants, the 1,673 healthy individuals averaged 8.97 leaves and the 605 mottled 4.76. The distributions of these two types overlapped considerably (table 5), but the differences between the two types were highly significant in every way that analyses were made, namely, on the basis of separate families, types of crosses, segregating ratios (table 5) and total population.

When these data on average number of leaves were grouped according to the expected ratios, analyses of variance showed highly significant differences between averages among both mottled and healthy plants (table 5). When the leaf number averages were compared with the average number of dominant factors expected in each of the classes, a consistent trend was evident. The more dominant factors carried by a class of plants, the lower was the average number of leaves. This inverse relationship between these seven pairs of averages is indicated by a value of $r = -0.944$, which is highly significant. Squaring this correlation coefficient indicates that 89 per cent of the variability in leaf number may be accounted for on the basis of the number of dominant genes borne by both mottled and healthy plants. In other words, despite the fact that all segregations based on leaf mottling were very clear cut between mottled and healthy plants (with no cases of intermediate type), there was superimposed on

this a highly significant dosage effect, which was expressed in the vigor of both mottled and healthy plants.

In all this material, growth in the seedling stage was slow and non-vigorous, with many plants failing to survive. In order to obtain the 4,193 plants classified in F_2, B, and F_3, a total of 6,376 seeds were sown. This represents a classifiable stand of 66 per cent. In almost all cases mortality occurred at an early seedling stage either before or after emergence from the peat and was not a result of poor germination. This was checked by careful observation of the seedling rows shortly after planting and in the case of the F_3 material by germinating the seeds on blotters in Petri dishes before planting in the peat. Nearly 100 per cent of the F_3 seeds germinated and were transplanted. The surviving stand, however, was only 68 per cent of the germinated seeds. In the B and F_2 populations, the stand was respectively 65 and 63 per cent of the total seeds sown. The differences between these per cents are not significant.

At first it was postulated that the poor stands might account for the deficiency of mottled plants, particularly in the families segregating 9:7 and 3:5. An analysis of the stand data, however, did not bear out such a conclusion. The total stands were not significantly different for the 5 types of segregations. Apparently there was some lethality among both healthy and mottled types and this tended to make the differences for different ratios non-significant. Additional evidence may be drawn from the F_2 and F_3 progeny tests, which showed respectively 1:1:4 ratios instead of 1:1:1 among the healthy plants and a 0:1:10 ratio instead of 1:4:4 among the mottled plants. Both healthy and mottled types showed a deficiency of plants which bore the greater number of dominant factors, but the deficiency was greater with the mottled plants. In other words, the lethality may have been proportionately more severe among the mottled plants in the 9:7 ratios than among some others, which might account for at least part of the deficiency of mottled plants. This could be checked by obtaining stands on the mottled and healthy types separately, but no such data were secured.

Other symptoms.—Mottling or flecking of the leaves (fig. 2) was the most characteristic symptom of the abnormal plants, but other symptoms were noticed in most abnormal individuals. The vegetative parts of affected plants were significantly fewer in number and generally were smaller in size. In addition the petioles of the abnormal plants often were twisted and bent, and the blades were partly inrolled (fig. 3). It should be pointed out that the differences shown in table 5, both between abnormal and healthy plants and between the segregating types within both classes probably would have been accentuated if some other measure of vigor, which would have taken into account both size and number (e.g., dry weight), had been used.

Cytological sections were prepared from typical leaves of both abnormal and healthy plants, but even with a variety of fixatives and stains it was difficult to observe the sequence of breakdown in the necrotic areas. Both palisade and spongy parenchyma layers appeared to be involved, since these cells were collapsed and stained heavily. In some of the lesions, palisade cells were not distinct, but it was difficult to relate this to a spreading of the necrotic areas.

Attempts at transmitting the abnormalities.—In order to obtain additional evidence concerning the nature of the virus-like injury, an effort was made to transmit it to healthy plants by mechanically inoculating them with the juice from affected plants. The healthy plants were dusted with carborundum powder and inoculated by the rubbing method. Inoculum was prepared by macerating mottled leaves in a sterile mortar with a few ml. of distilled water. A sterile square of folded gauze was dipped in the inoculum and gently rubbed over leaves supported in the hand. After inoculation, the plants were sprinkled with tap water to remove possible toxic substances. In the same manner, juice from healthy leaves was used to inoculate controls. The inoculated plants were incubated in a greenhouse at 20° to 25°C. Observation of the inoculated plants was continued for several months, but none of them showed evidence that the abnormal condition was transmitted through mechanical inoculation.

An attempt was then made to transmit the virus-like injury to peas by mechanical inoculation. Several of the varieties tested by Johnson (1942) were used, including Potlatch, Nott's Excelsior, Little Marvel and Hundredfold. The plants of each variety were grown from seed in a greenhouse during the winter and inoculated by the rubbing method when approximately four inches tall. In addition to inoculating peas with juice from plants having virus-like symptoms, peas of each variety were inoculated with juice from leaves of white clover known to be infected with mosaic. Control plants of each variety of peas were inoculated with juice from healthy leaves of white clover. All inoculated plants were rinsed with water and incubated at greenhouse temperatures of 20° to 25°C. The plants were observed daily, and final notes were taken 21 days after inoculation. None of the peas inoculated with juice from leaves of white clover with the virus-like injury showed evidence that the disorder was transmissible. From 50 to 100 per cent of the peas inoculated with juice from known mosaic-infected plants of white clover were either dying or showing severe symptoms of virus infection. None of the controls were diseased.

Since no mechanical transmission of the virus-like injury resulted, an effort was made to transmit the abnormality by grafting stolons of healthy and mottled plants. Best union of stock and scion resulted when a slit-type graft was used. In order to prepare the scion, a vigorous stolon with young, unfolded leaves at the apex was severed approxi-

mately 1/16 inch below the second node from the tip. The cut end of the stolon was trimmed to a wedge-shape by making two diagonal cuts through the basal node with a razor blade. The stock was prepared by cutting off a stolon slightly anterior to any nearly-terminal node. A slit was made lengthwise through the center of the stock node, and the wedge-shaped scion was inserted into the slit. Joining nodes of stock and scion in this manner permitted union between cut surfaces of meristematic tissues. Different methods of protecting the grafts were tried, but greatest success resulted by simply covering the graft with a clover leaflet or a small amount of moist peat moss. Reciprocal grafts were made between all plants tested. Of 52 grafts attempted, 13 survived for one month or more. At the end of eight months, 8 of the grafts were producing vigorous growth with none showing evidence that the abnormal condition was transmitted either from stock to scion or vice versa by grafting. This lent additional support to the hypothesis that the virus-like symptoms were genetic in character.

Discussion.—This heritable virus-like injury of white clover is of interest because it is a well defined case of genetic factors producing injury similar to that caused by a virus infection. It seems reasonably certain that genes rather than virus were involved since the genetic data were consistent with a two factor hypothesis. In addition none of the attempts at transmission were successful, whereas with the common types of white clover virus these same methods of transmission provide easy means of transfer.

The observations on leaf number as a measure of vigor provided an interesting corollary to the main genetic observations. The differences in average number of leaves were highly significant not only between mottled and healthy plants but also between the different segregating ratios within each type. These latter differences were inversely correlated with the average number of dominant genes borne by the different classes of plants. Similar clear-cut cases of dosage effect are common in genetic literature, but in general the genes which produce such an effect do not condition other phenotypic segregations. In this case lack of vigor was one of the abnormal symptoms, and its multiple segregation due to dosage effect was superimposed on the sharply bilateral segregation observed through the principal symptom of presence or absence of mottling. Both the vigor effect and the mottling effect were caused by the same genes. This indicates that genes influencing a specific character also have a measurable effect on the general quantitative characters of the plant.

SUMMARY

A selected plant of white clover, which showed severe mottling of its leaf blades similar to the symptoms caused by virus, was crossed with an unrelated healthy plant, and the F_1 segregated into 12 mottled and 33 healthy.

A total population of 4,193 plants from various B (backcrosses), F_2, and F_3 families was classified in the greenhouse. All segregations were well defined, with no cases of intermediate injury.

Ratios of 9:7, 3:1, 3:5, 1:3 and no segregation were obtained in different families, depending on the genotypes involved. Most of the obtained ratios fitted the expected closely, but in general there was a deficiency of mottled plants, particularly in the families segregating 9:7 or 3:5.

These results are explained by assuming two independent dominant factors, both of which must be present for the development of the necrotic lesions.

Evidence from B, F_2, and F_3 families suggested that the abnormal and healthy plants bearing the largest number of dominant factors tended to be lethal. No significant differences in stand were obtained, however, between the several segregating ratios.

The number of leaves at the age of six weeks was used as a measure of vigor; the 1,673 healthy B and F_2 individuals averaged 8.97 leaves per plant and the 605 abnormal plants 4.76; the difference between these averages was highly significant.

The differences in average number of leaves were also highly significant between the different segregating ratios among both healthy and abnormal types. These averages were inversely correlated ($r = -0.944$) with the number of dominant factors borne by the different classes, indicating a highly significant dosage effect caused by the same genes that conditioned the mottling but with the dosage effect superimposed on the sharply bilateral segregation observed with presence or absence of mottling.

All attempts to transmit the mottling by inoculating or grafting gave negative results, giving further evidence that the agent in this case was not a virus.

This condition is of interest since it is another example of the fact that some heritable factors may induce types of morphological responses similar to those induced by certain disease agents.

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AN ANALYSIS OF THE FUNCTION OF THE LEAF IN THE PROCESS OF ROOT FORMATION IN CUTTINGS [1]

J. van Overbeek, Solon A. Gordon, and Luis E. Gregory

IN PREVIOUS papers (van Overbeek and Gregory, 1945, and Gregory and van Overbeek, 1945), it was shown that for the formation of roots on cuttings of a difficult-to-root hibiscus variety two distinct factors are necessary. One of these factors is auxin, which was supplied to the cuttings by dipping their bases in an alcoholic solution of indolebutyric acid (hereafter referred to as IB). The other factor was found to be present in the leaves of the easy-to-root red hibiscus and was given to the cuttings by grafting a shoot of red hibiscus on as a donor.

The present paper deals with attempts to analyze this root-promoting effect of the leaves and to investigate its chemical nature.

In the course of the work it soon became apparent that cuttings of red hibiscus, without grafting, also showed the root-forming effect of leaves. Here also, both leaves and auxin were found to be necessary for root formation (fig. 1, 2). This simplified the analysis of the root-forming factors of the leaves considerably. Leafless "sticks" could now be used as indicators or testers for the root-inducing activity of various substances which were applied together with IB.

In the absence of auxin none of the many compounds tried showed any activity. However, in the presence of auxin two groups of chemicals showed significant root-forming activity. These substances are sugars and both organic and inorganic sources of nitrogen. A combination of IB, sucrose and a suitable source of nitrogen could entirely replace the leaves in so far as number (not quality) of roots formed was concerned. Each of these substances by itself is ineffective, just as either leaves or IB by themselves are ineffective.

In order to determine if the types of substances which could replace the action of the leaves were also actually contributed by the leaves, chemical analyses of the base of leafy and defoliated cuttings were made. The results obtained confirmed this assumption.

METHODS.—The main experiments were performed with cuttings of the common red *Hibiscus rosa-sinensis* L. of which an abundant supply of relatively uniform quality was available throughout the year. The cuttings were of the semi-hardwood type, 3 to 5 mm. in diameter. The soft terminal part of the shoots was removed, in order to avoid complications, as this part is in all probability a source of natural auxin (Went and Thimann, 1937; van Overbeek and Gregory, 1945). The length of these cuttings depended upon the type of experiment to be performed and ranged from three to twelve inches. The type of cuttings used is shown in figures 1, 2, and 6.

Auxin applications were made by the alcohol dip method (Hitchcock and Zimmerman, 1939; Cooper, 1944). In most experiments this consisted of dipping the base of the cuttings in 50 per cent alcohol containing 2 mg. of IB per cc. In some experiments the concentration was varied, while in others the auxin application was made at the apex rather than at the base of the cutting. These variations are noted below.

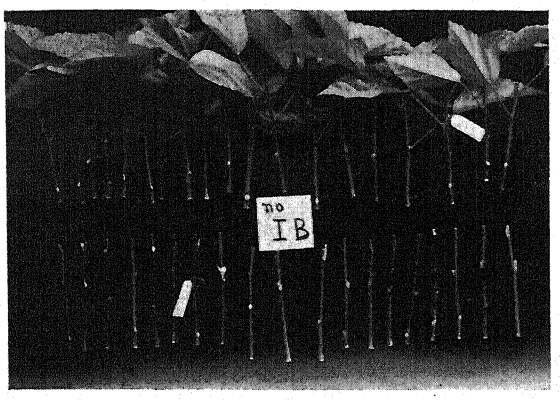
When treatments with various substances other than auxin were given, these compounds were dissolved in water, and the base of the cuttings then soaked in the solutions for 24 hours. Treatments of this type were followed by an IB dip, if the latter was required. In these tests the length of the leafless cuttings was usually three inches. This length was selected because these short cuttings contain less reserve material than longer ones (table 1).

TABLE 1. *Number of roots formed on defoliated red hibiscus cuttings as a function of cutting length. Averages of 20 cuttings.*

Length of cuttings (inches)	3	5	6	12
IB, 2 mg./cc. at base.....	1.6	..	4.1	7.7
	0.1	1.5	..	5.2
No IB	0	..	0	0
	0	0	..	0.7

After treatment the cuttings were placed in moist sand of a propagating frame. This structure was of the general type used at the Imperial College of Tropical Agriculture at Trinidad (Cheesman and Spencer, 1936). It was glass-covered with overhead shades constructed of bamboo. The incident light intensity at the sand in the frames at midday

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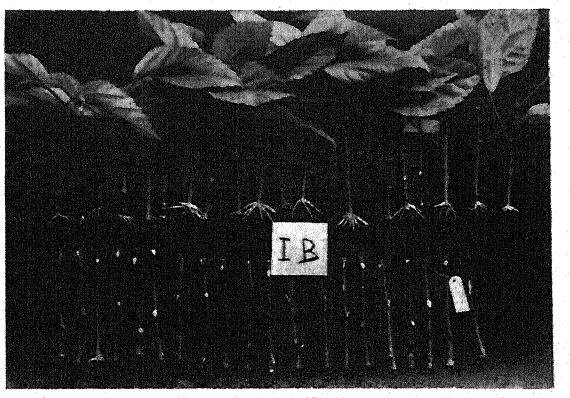


Fig. 1 (left) and 2. Root formation on red hibiscus cuttings as affected by the presence of leaves and treatment at the base with 2 mg./cc. indolebutyric acid (IB) by the alcohol dip method. Only when leaves and auxin are both present is a significant number of roots formed. Cuttings five inches long. Photographed 14 days after treatment. Note that cuttings have no terminal bud.

without clouds amounted to approximately 500 foot candles. In several special experiments the glass was covered by black tar paper. The temperature of the sand in the frame was approximately 25°C.

Root counts were made 14 days after the cuttings were set in the sand. For chemical analyses the cuttings were removed after periods ranging from three to nine days. At these times no roots were externally visible.

For sugar assays the colorimetric ferricyanide method suggested by Morell (1941) was adapted for the use of a Coleman Model 11 spectrophotometer. Nonreducing sugars were determined after inversion by acid hydrolysis and were calculated as glucose. For the estimation of nitrogen the technique of Lindner (1944) was modified for use of the above instrument.

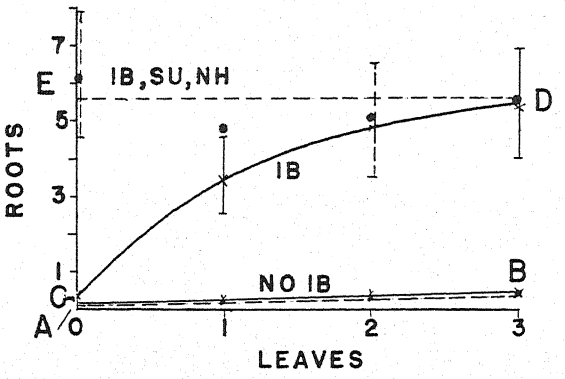
RESULTS.—*Relation between auxin and rooting.*—The work on the grafting of red and white hibiscus, mentioned at the beginning of this paper, showed clearly that for the rooting of white hibiscus a combination of auxin and leaves is necessary. This same principle also holds for red hibiscus cuttings without grafting (fig. 1, 2, 3), for *Allamanda cathartica* L. (fig. 4), for acalypha (van Overbeek and Gregory, 1945), for bougainvillea, roses, gardenia and for coffee (van Overbeek *et al.*, 1944–45). It appears to be a general principle.

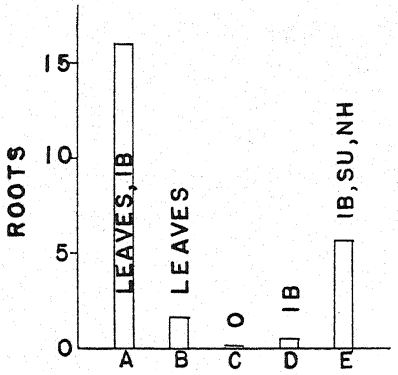
Without auxin treatment and without leaves no roots were obtained in red hibiscus cuttings (fig. 3, point A). When leaves were present but without auxin treatment, the number of roots formed was still insignificant, as is shown by curve AB (solid line). However, when both leaves and IB are present, the number of roots increases with an increasing number of leaves, as is demonstrated by curve CD. The remaining curves of figure 3 will be dealt with below.

In figure 4 the same principle is demonstrated for *Allamanda cathartica* L. Column C shows that untreated cuttings without leaves formed no roots, while auxin-treated cuttings without leaves also produced an insignificant number (D), as did cuttings with leaves alone (B). But when both auxin and leaves were present, an average of 17 roots per cutting was formed (A).

Leaves are effective in darkness.—After we began to understand the mutual effects between leaves and auxin in the process of root formation, the question arose as to how light would affect the root-inducing ability of leaves. It developed that a concurrent exposure to light is not necessary for leaves to exert their root-inducing effect; leaves are effective equally well in darkness as in the subdued light of the propagating frame. This is shown in figure 5, which gives the results of an experiment similar to that described by figure 3. However, in the present experiment one of the two sets of cuttings was kept in a dark propagating frame. This frame was light-tight and was only briefly opened for sprinkling once every two days. The hibiscus cuttings retained their leaves well in the dark during the period of 14 days required to complete the test.

Similar experiments were conducted with *Allamanda cathartica* L., and, although the leaves of the cuttings in the dark frame did not stay on for the whole 20 days that the experiment lasted, the root-inducing effect of leaves was unmistakable. Without IB treatment no roots were formed on leafless cuttings nor on leafy ones. Only an average of 0.9 roots per cutting was produced in leafless cuttings with IB, but an average of 8.2 roots per cutting was formed on cuttings which had been placed in the dark propagating frame while possessing leaves and which in addition had received an auxin treatment. Similar results were obtained with three varieties of bougainvillea. It seems, therefore, that the root-inducing factors of the leaf can remain effective even though further photosynthetic activity ceases. Either these factors may arise from reserve substances, or they are present in sufficient amounts to supply the stem during a prolonged period of darkness.





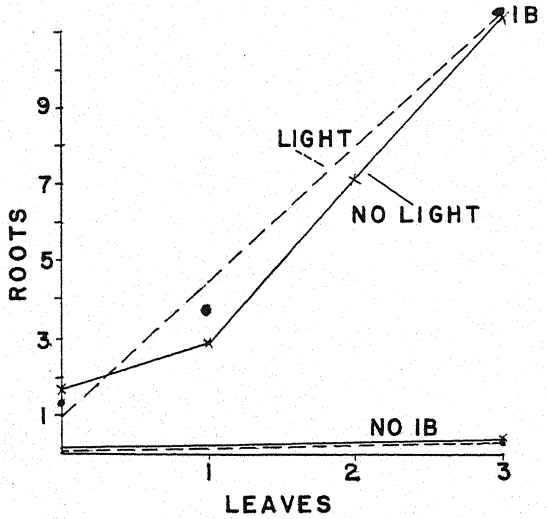


Fig. 3–5.—Fig. 3 (above). Average number of roots formed on red hibiscus cuttings as affected by the number of leaves per cutting, by auxin treatment (IB, 2 mg./cc. at the base), and by treatment with 4 per cent sucrose (SU) and 0.1 per cent ammonium sulfate (NH). Broken lines: SU and NH treatments. Solid lines: without SU and NH. Averages of 20 cuttings. Vertical lines indicate range of standard errors.—Fig. 4. Average number of roots formed on allamanda cuttings as affected by the presence of three to four leaves, by treatment with IB at the base, and by a combination of sucrose (4 per cent) and ammonium sulfate (0.1 per cent). Observations 20 days after treatment.—Fig. 5. Average number of roots formed on red hibiscus cuttings as affected by the number of leaves, and by treatment with IB at the base. One experiment took place in the indirect daylight of a regular propagating frame, while another test took place in a dark propagating frame. The curves show that light is not directly necessary for the root-promoting function of leaves.

Substances which replace the leaf in root formation.—After it had become clear that cuttings of the red hibiscus produce as a rule only an insignificant number of roots—even when treated with auxin—unless leaves are present (fig. 1, 2, 3, 4, 5), the way was paved for a practical test for root-forming substances other than auxin. It is only necessary to test leafless, auxin-treated cuttings with substances suspected of having root-forming activity.

After a variety of compounds was tried[2], it became clear that two groups of substances had an outstanding ability to produce roots, but only when tested in combination with auxin. These groups are sugars and nitrogenous substances. In the first group sucrose is effective (fig. 8, 12). In the second group one can distinguish between inorganic and organic sources of nitrogen. Ammonium sulfate is outstanding among the inorganic compounds (fig. 7), while arginine (fig. 8) is an example of an active compound of the other group. The nitrogenous substances show their activity best in the presence of sucrose (fig. 8). The main difference between organic and inorganic sources of nitrogen was that organic substances such as arginine caused an optimum effect in concentrations of 10 mg. per liter when applied by soaking the base of the cuttings for a period of 24 hours. When ammonium sulphate was used, the optimum effect was obtained with concentrations 100 to 500 times higher (fig. 1, 7, 12).

One now may turn again to figure 3 and consider point E which represents the number of roots produced on leafless cuttings which had been treated with IB as well as sucrose (4 per cent) and ammonium sulfate (1000 mg./l.). It will be clear that this combination of chemicals produced a number of roots which was equal to that produced by three leaves (point D). The curve ED shows that the effects of sucrose plus ammonium sulfate and leaves are not additive. The curve AB (broken line) shows that sucrose and ammonium sulfate in the absence of IB are not effective, either without leaves (point A) or in the presence of leaves.

[2] The following organic substances gave a positive response in two or more tests when tested in concentrations between 1 and 100 mg./l. in the presence of IB and sucrose: arginine, betaine, choline, and serine. The following organic substances gave either no response or no significant response: ascorbic acid, aspartic acid, asparagine, adenine sulfate, para-amino benzoic acid, biotin, cysteine, cystine, fumaric acid, glutamic acid, glycine, histidine, hydroxyproline, inositol, isoleucine, l-leucine, nor-leucine, methionine, pimelic acid, phenyl alanine, succinic acid, tryptophane, threonine, l-tyrosine, 2, 3, 5 tri-iodobenzoic acid, urea, uric acid.



Fig. 6. Root formation on red hibiscus cuttings under the influence of auxin (IB, 2 mg./cc.) and leaves, as compared to root formation under the influence of IB, 4 per cent sucrose and 0.1 per cent ammonium sulfate. The number of roots formed in the upper row is 6.2 ± 1.6, that in the lower row 4.8 ± 1.1. Although sucrose and ammonium sulfate replace the action of leaves entirely as far as number of roots is concerned, the quality of the roots in the leafy cuttings is better. The cuttings shown here belong to the same series as those of figure 3.

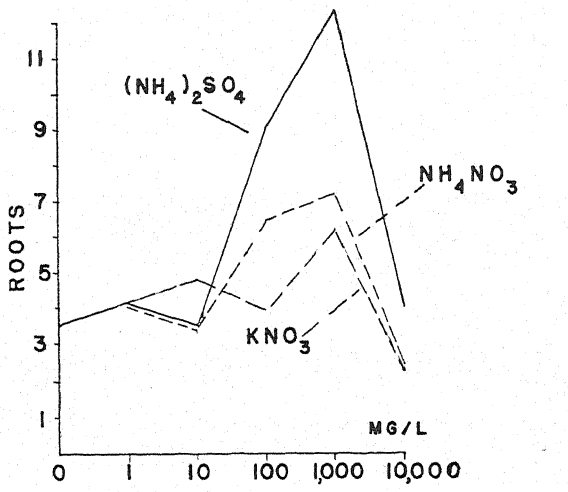
The effect of arginine is shown in the diagrams of figure 8. This experiment was carried out in the dark propagating frame, and the IB was applied at the apical part of the cuttings. With IB only (column B) a number of roots was produced which was about one-third of that produced by three leaves plus IB (column A). In cuttings treated with both auxin and sucrose (C), the number of roots was somewhat increased over those treated with IB alone. IB and arginine in concentrations ranging from 1 to 50 mg./l. (D) produced about the same number of roots as IB and sucrose. A combination of IB, sucrose, and arginine at a concentration of 10 mg./l. (E), caused the formation of a number of roots equal to that produced by IB plus three leaves.

In the presence of sucrose and auxin, a combination of choline, serine and arginine (G) produced results similar to that of arginine (E). Both choline and serine in a concentration of 10 mg./l. gave increased root formation when tested with IB and sucrose as previously mentioned. But these substances did not reach "leaf level" as did arginine and ammonium sulfate.

Occasionally there appeared to be so much reserve substance in the stems of red hibiscus that leafless cuttings rooted profusely with auxin treatment alone. Such cuttings showed no additional effect of sucrose, of nitrogenous substances, and at times even of leaves. There are indications that this condition may fluctuate with the seasons. In our search for methods which would eliminate this disturbing effect, it was found that the shorter the length of the cutting, the less was the effect of reserve substances (table 1). For this reason, cuttings of only three inches long were used in the routine tests, shorter lengths being too difficult to handle.

It may also be pointed out that, although the *number* of roots in the above-mentioned tests is the same in auxin-treated cuttings with leaves as in auxin-treated cuttings in which the leaves had been replaced by a combination of chemicals, the *quality* of the resulting roots is considerably better in the leafy cuttings. It is quite possible that additions of root growth factors, such as some of the B vitamins (Robbins and Kavanagh, 1941; Bonner, 1940; White, 1943), may produce a root system which is more comparable to that produced by leaves.

The root-inducing effect of sucrose and nitrogenous substances, which has been detailed above for leafless cuttings of red hibiscus, also holds for cuttings of the two other species which were tried. Figure 4 (E) shows that, in leafless cuttings of allamanda which were treated with sucrose and ammonium sulfate in the presence of IB, the number of roots formed was considerably increased over that in cuttings treated with IB alone (D), even though the number of roots formed was considerably below that of IB-treated leafy cuttings (A).



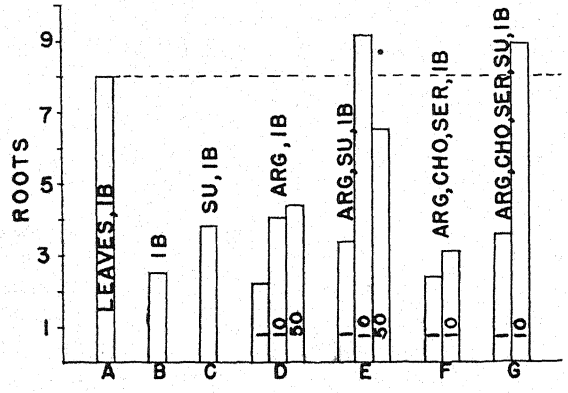


Fig. 7–8.—Fig. 7 (above). Average number of roots formed on defoliated red hibiscus cuttings as affected by various concentrations of inorganic nitrogen compounds (abscissa). All cuttings were treated with 4 per cent sucrose and 2 mg./cc. IB; O on abscissa refers to IB plus sucrose without nitrogen. Observations 18 days after treatment.—Fig. 8. Average number of roots formed on defoliated red hibiscus cuttings as affected by organic nitrogen compounds in combination with 4 per cent sucrose and 2 mg./cc. of IB applied at the apex. Comparable leafy cuttings treated with IB are shown in column A. ARG, arginine; CHO, choline; SER, serine. The figures in the bars indicate the concentration of these substances in mg./l. A dark propagating frame was used.

Recently, similar results have been obtained with thin leafless cuttings of white hibiscus and white bougainvillea.

Analyses of sugar and nitrogen in cuttings.—It was shown above that the action of leaves in the process of root formation can be replaced by sucrose in combination with a suitable source of nitrogen. In order to ascertain whether or not the leaves actually contribute these substances, a series of analyses was made of the basal part of leafy and defoliated cuttings. Figures 9 and 10 show that both reducing and non-reducing sugars in the base of the cutting increase with the number of leaves. The curves resemble those of figures 3 and 5 where the number of roots was directly controlled by the number of leaves. The sugar curves of cuttings which were kept in the light show the same trend as those of cuttings kept in a dark propagating frame. As could be expected, the absolute amounts of sugars are larger in the former group.

Figure 11 shows that the amount of soluble nitrogen increases in the base of the cuttings as a function of the time that they were in the (dark) propagating frame. This increase is considerably stronger in leafy than in defoliated cuttings.

It will be noted from figures 9, 10 and 11 that leafy cuttings which had been treated with IB showed a higher sugar as well as nitrogen level than the untreated controls. The data of these figures are expressed on a dry weight basis; no significant change in trend was noted when these data were plotted on a green weight basis. In a future publication we hope to elaborate on the marked effect of IB on the sugar and nitrogen levels in the base of the cuttings.

Proof that actual root initiation is involved.—At a time when so much mention is made of special root-forming substances, the fact that sucrose and the relatively simple nitrogenous substances can entirely replace the leaf as far as the actual formation of roots is concerned is of interest. In order to investigate the possibility that auxin initiates the roots which sucrose and nitrogenous substances subsequently cause to grow out, thus only causing an apparent effect of sucrose and nitrogenous substances on root formation, thousands of sections of the basal part of cuttings of red hibiscus were examined and the root initials counted. The cuttings were sectioned by free hand methods and examined under a Bausch and Lomb binocular preparation microscope (20 X). The bark was removed from the wood in order to facilitate sectioning. The results of one experiment are given in figure 12. These data show that the untreated cuttings possess no root initials (column A), and that the number of initials increases considerably when IB treatment in combination with sucrose (D), and sucrose (4 per cent) plus ammonium sulfate (0.5 per cent) (E) is given, as compared to those cuttings treated with IB alone (C).

It is interesting to observe that, though the number of externally visible roots (cross-hatched part of columns) of (C) and (D) did not differ significantly in this experiment, the difference between (C) and (D) became statistically highly significant when the initials were counted. This shows plainly that it is root initiation itself—and not merely root elongation—which is stimulated by sucrose, and also by ammonium sulfate (E) in auxin-treated cuttings.

Origin of the root initials in red hibiscus.—As a result of the above mentioned microscopic examination of cuttings, it was found that the root initials, induced by IB and additional substances in red hibiscus cuttings, originate in the secondary phloem

(bark) in the ray parenchyma. This is in agreement with the usual pattern of root initiation shown in stems in which the cambium is active (Priestley and Swingle, 1929; Hayward, 1938).

Discussion.—The main purpose of the work presented here was to learn more about the role of leaves in the process of root formation in cuttings. It has become clear that the action of mature leaves on root initiation is different from that of auxin. However, leaves will not exert their root-inducing effect without auxin. Conversely, auxin exerts its effect only in conjunction with substances supplied by the leaves.

In recent years (Went, 1938) it has been suggested that besides auxin another hormone is necessary for root formation. This hypothetical substance was called "rhizocaline" and was considered to be a specific root-forming hormone which is produced in leaves. Our experiments with hibiscus and other tropical plants leave no doubt that besides auxin something additional is necessary for the production of roots. There is also no doubt that this other requisite comes from the leaves. So far, but no further, our data agree with the caline concept. The experiments discussed above have shown clearly that the effect of leaves is exerted equally well in darkness and in light. This is at variance with the caline concept, according to which rhizocaline is produced in the light (Went, 1938). Furthermore, the root-forming effect of the leaves could be entirely replaced by non-specific substances such as sucrose in combination with ammonium sulfate or arginine.

Chemical analyses of the base of the cuttings showed that the sugar content is considerably higher in cuttings with leaves than in defoliated ones. This effect was found in the light as well as in darkness. Analyses of soluble nitrogen showed the same. Data to be published later showed that insoluble nitrogen also increases in the base of the leafy as compared to defoliated cuttings, showing that the increase in soluble nitrogen was not solely the result of proteolysis in the cutting base. From the above, therefore, it is reasonable to conclude that the chief effect of the leaf in the process of root formation is through its supply of nutritional factors to the base of the cutting.

Since with these above-mentioned substances a number of roots could be obtained which was equal to that initiated under influence of leaves, there is no *a priori* reason to assume that additional highly specific substances necessary for the *initiation* of roots are produced by the leaves. A glance at figure 6, however, will show that, even though the number of roots of the sucrose and ammonium sulfate-treated cuttings (6.2 ± 1.6) somewhat exceeded that of the leafy ones (4.8 ± 1.1), the quality of the roots is better in the latter series. This makes it probable that the mature leaves contribute certain root *growth* factors. Bonner (1942) concluded that thiamine is produced by the mature leaf. Later work (Bonner, 1944; Burkholder and McVeigh, 1945) shows the production in shoots of other nutrilites, some of which are known to be essential for root growth *in vitro* (Addicott, 1941; Bonner, 1940; Robbins and Kavanagh, 1941; White, 1943).

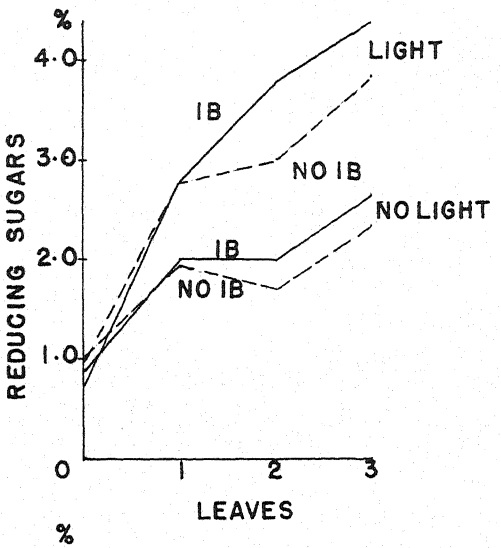
Although on the basis of the hibiscus experiments it may seem unlikely that the leaves contribute a special "rhizocaline" to the cuttings, the possibility is nevertheless not excluded that there exist specific or non-specific root-forming substances other than auxin and nutritional factors. If such substances exist, they may have been present in the reserve substances in the stem, and become activated by IB and/or sugar and nitrogenous substances, or they may have been produced at the base of the cutting as a result of the combined action of these substances. Under certain conditions they may be produced in leaves. Except for some preliminary experiments which indicate that some further non-specific substances may be involved in root formation, we do not have at present any definite evidence in favor of, or in opposition to, the above possibilities.

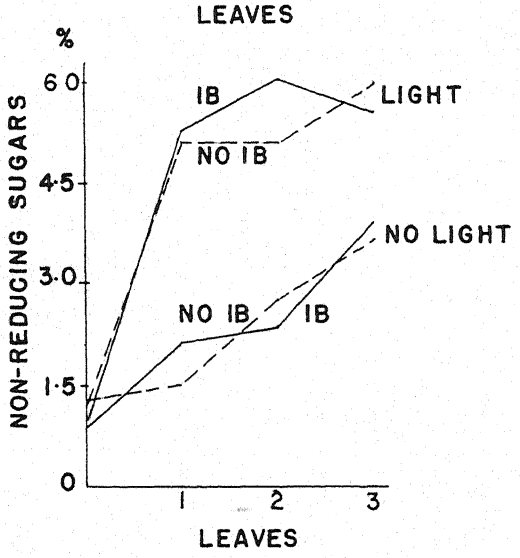
Although, as far as we are aware, no successful experiments have been reported in which the effect of leaves in root formation was completely replaced by known compounds, there are reports of increased root formation by substances other than auxin. Thus, Doak (1940) found that in rhododendron cuttings no roots are produced with the auxin naphthaleneacetic acid alone, but that this substance produced roots when applied in combination with both organic and inorganic nitrogen compounds. Ammonium sulfate and arginine are among the active substances. Doak suggests that these nitrogenous chemicals have a similar effect as the postulated "rhizocaline." Our results are in complete agreement with those of Doak.

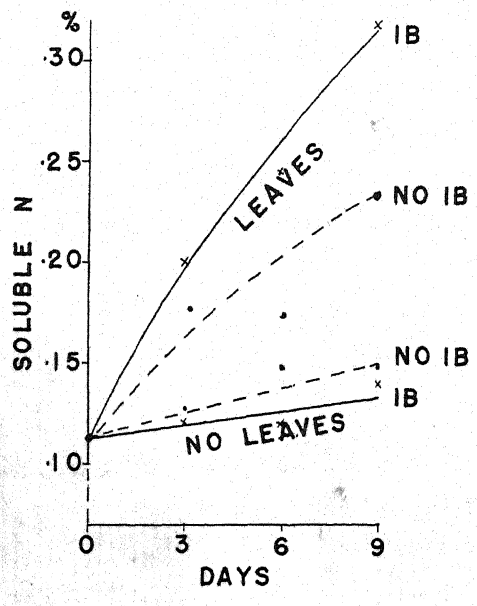
Beal (1940) found that small isolated sections of the stem of bean plants are capable of producing roots when treated with the auxin indoleacetic acid, provided they are kept on a complete nutrient solution. When either the sugar or the nitrogen source is omitted from the medium, no rooting takes place. Our experiments also agree with these observations.

Thimann and Poutasse (1941), although they believed that they were dealing with the outgrowth and maintenance of roots rather than their initiation, also found that nitrogenous materials are of importance for the root formation of bean cuttings. Their experiments differ from ours in that the substances they used, among which was choline, are capable of causing an increased root formation over that of the control without added auxin. In view of the nature of their material it may be assumed, however, that this contained an ample supply of native auxin.

Sugars, with and without additional auxin treatment, have been tried by numerous investigators. For practical purposes we have found that for leafy







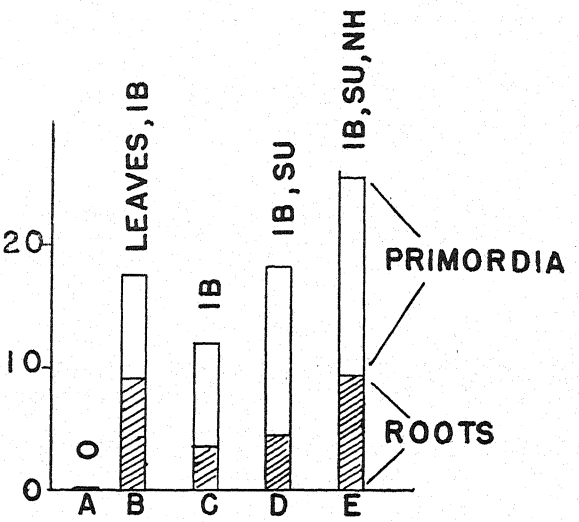


Fig. 12. Average number of roots (shaded columns) and root primordia (white columns) formed on red hibiscus cuttings as affected by a variety of treatments. IB was applied at the apical end of the cuttings. SU, sucrose 4 per cent; NH, ammonium sulfate 0.5 per cent. The figure shows that the treatments cause actual formation of roots and not merely the growing out of existing primordia.

cuttings a sucrose treatment followed by an IB dip is beneficial in some cases (gardenia, citrus). Nitrogen treatments of cuttings, on the other hand, often produce inhibitory effects, so that in practice such treatments have to be applied with caution. Retention of the leaves is of prime importance in the successful rooting of the majority of cuttings, especially of the more difficult-to-root species (van Overbeek *et al.*, 1944–45).

SUMMARY

The combined action of auxin and factors coming from the leaves is essential for root formation in cuttings (fig. 1, 2, 3, 4). The leaves exert their influence in the dark as well as in the light (fig. 5). In red hibiscus the root-forming factors of the leaves can be entirely replaced by sucrose and nitrogenous substances (fig. 3, 6, 8). Quantitative chemical analyses show that these substances are actually contributed to the cuttings by the leaves (fig. 9, 10,

Fig. 9–11.—Fig. 9 and 10. Effect of the number of leaves on the sugar content of the basal two inches of red hibiscus cuttings. Tests were made nine days after treatment and were calculated on a dry weight basis. One set of experiments was conducted on cuttings kept in the light of a regular propagating frame, while another set was conducted on cuttings kept in the dark. Fifty cuttings were used for each determination.—Fig. 11. Increase of soluble nitrogen in the basal 1.5 inches of red hibiscus cuttings as a function of the time (abscissa) after the cuttings were placed in the propagating frame. Cuttings with two leaves show a more marked increase than defoliated ones. Averages of two experiments, in which 75 cuttings were used for each determination. The cuttings of one of these series are shown in figures 1 and 2.

11). It is, therefore, concluded that the main function of leaves in the process of root initiation is to supply the cutting with sugars and nitrogenous substances, factors which may be termed nutritional. There is no *a priori* reason to postulate the production of a special root-forming substance such as "rhizocaline" in the leaves of red hibiscus.

In red hibiscus cuttings, after treatment with auxin in conjunction with leaves or with suitable carbohydrate and nitrogen sources, roots were found to originate in the secondary phloem in the ray parenchyma.

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THE ABSORPTION BY ROOTS OF RUBIDIUM AND PHOSPHATE IONS AT EXTREMELY SMALL CONCENTRATIONS AS REVEALED BY EXPERIMENTS WITH Rb^{86} AND P^{32} PREPARED WITHOUT INERT CARRIER [1]

Roy Overstreet and Louis Jacobson

THE STUDY of ion-absorption by roots from a soil is obscured by innumerable root-soil relationships. As a consequence, recourse generally has been made to the study of simpler systems; that is, of plants and excised roots in artificially prepared solutions, and by these means considerable information has been collected concerning the ion-absorption process. In this connection, the use of excised barley roots has been of particular importance (Hoagland and Broyer, 1936, 1940; Hoagland, 1940).

An important aspect of the ion-absorption process, as revealed by past experiments, is its dependence on growth and on the active metabolism of the plant. An abundance of evidence points to the fact that the accumulation of either cations or anions by plant roots proceeds concomitantly with such processes as respiration, reduction of sugars, synthesis of organic acids, etc. (Hoagland and Broyer, 1936, 1942). When the metabolic processes are inhibited by poisons, oxygen deficiency or lowered temperature, no accumulation takes place. This dependence

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Division of Soils and Division of Plant Nutrition. The authors are indebted to the staff of the 60-inch Cyclotron, Crocker Radiation Laboratory, University of California, for the production of the radio-rubidium and radio-phosphorus.

of the process on metabolic activity has been brought out with especial clarity by studies with unbranched roots where it is found that the greatest absorption occurs near the root tip (Prevot and Steward, 1936; Steward, Prevot and Harrison, 1942; Machlis, 1944).

Under conditions favorable for growth, the rate of absorption of an anion or cation by plant roots is to some degree dependent on the concentration of the ion in question in the culture medium, provided a certain limiting value is not exceeded. For example, the rate of absorption of K^+ by "low-salt" barley roots was found to be dependent on the K^+ content of the external solution up to concentrations of approximately 5 milliequivalents per liter, but the absorption rate was not increased by greater concentrations (Overstreet *et al.*, 1942).

A further salient characteristic of the absorbing root system is the apparently independent entry of cations and anions from the culture solution into the root. For example, with K_2SO_4 culture solutions, the accumulation of K^+ by barley roots is much greater than the accumulation of $SO^{=}_4$. Likewise, in the case of $Ca(NO_3)_2$ solutions, the accumulation of NO^-_3 far exceeds the accumulation of Ca^{++} (Hoagland, 1940). An excess accumulation of anions over cations is usually compensated in the culture solution by HCO^-_3 ions, and an excess accumulation of cations over anions is compensated by H^+ and other cations such as Na^+, Ca^{++} and Mg^{++} from the plant. Observations of this nature lend support to the theory that the initial step in the ion-absorption process is an exchange adsorption (Brooks, 1937). According to this idea, cations of the culture medium are fixed by the root in exchange for H^+, Ca^{++}, Na^+, etc., initially present in the protoplasm. Similarly, anions are fixed in exchange for HCO^-_3 and possibly other anions.

Recently the study of the mineral nutrition of plants has been greatly facilitated by the use of artificially prepared radioactive elements. This is so because their radioactivity distinguishes the entrant ions from all those which are present in normal roots and permits them to be determined with an extreme degree of sensitivity by means of the Geiger-Müller counter. One of the many uses of radioelements has been their employment in the study of exchange reactions between roots and their environment. By these means it has been possible to demonstrate ionic exchanges between the culture medium and plant roots involving both isotopes of the same element as well as ions of different elements (Jenny, Overstreet, and Ayers, 1939). A notable feature of these ionic exchange reactions is their apparent independence of the metabolic activity of the plant. The reactions readily occur in the neighborhood of 0°C., at which temperature metabolic activity (respiration, etc.) is very low (Broyer and Overstreet, 1940).

As far as the authors are aware, the radioelements hitherto used in the study of plants have always been accompanied by a preponderance of the inert elements; the radioactive isotopes serving merely as a "tag" for the inert isotopes. In radioactive phosphorus preparations as ordinarily used, the radioactive atoms (P^{32}) constitute an insignificant fraction of the total phosphorus atoms present. Preparations of this type are known in radiochemistry as "radioelements prepared with inert carrier." In recent years, methods for the isolation of certain radio elements *without* inert carrier have been developed. For example, radioactive Rb can be prepared in the cyclotron by transmutation from Sr (Helmholtz, Pecher, and Stout, 1941). The major part of the Rb atoms so prepared are radioactive. By suitable methods of separation, the Rb* atoms can be separated out from the Sr and other contaminating elements in the form of a solution. Solutions of this type, although often highly radioactive, are extremely dilute; the concentration of the ions being of the order of 10^{-9} mols per liter.

The behavior of radioelements prepared both with and without inert carrier has been studied in the field of animal physiology. It has been observed that certain basic processes are often revealed with the use of radioelements without inert carrier which are obscured when macro amounts of the elements are used. An instance of this is the behavior of iodine in the body (Hamilton, 1942). Hence, certain aspects of ion-absorption by plants may well be revealed by the use of radioelements prepared without carrier, which hitherto have been masked by the necessary use of the absorbed ions in macro amounts.

The experimental part of this paper is devoted to a study of the behavior in plant roots of two ions (Rb^+ and $PO^{=}_4$) both of which have been isolated without the addition of inert carrier. The experiments were performed with single excised barley roots and particular attention was paid to the phenomenon of exchange adsorption.

EXPERIMENTAL.—Both the radio-rubidium and radio-phosphorus were prepared by means of the 60-inch cyclotron in the University of California Radiation Laboratory.

The Rb isotope used was Rb^{86} (half-life = 19.5 days). It was prepared by a deuteron bombardment of Sr metal according to the nuclear reaction which is represented by the following equation in which atomic weights are written in the position of "exponents" to the elementary symbol and the lower series of numbers denote atomic numbers.

$$\underset{38}{\overset{88}{Sr}} + \underset{1}{\overset{2}{H}} \longrightarrow \underset{37}{\overset{86}{Rb}} + \underset{2}{\overset{4}{He}}$$

Radio-Sr, radio-Y, radio-Cu and radio-Zn (the last two elements from the Cu target plate) are also produced by the bombardment. Y is removed as the hydroxide and Sr, Cu and Zn as the carbonates, using appropriate "holdback" carriers. The purity of the final radio-Rb solution was checked by a determination of the decay curve. No detectable radioactive impurity was found.

Radio-phosphorus was prepared by neutron irradiation of sulfur in the 60-inch cyclotron. The radioactive isotope produced is P^{32} (half-life = 14.3 days). The nuclear reaction is as follows:

$$^{32}_{16}S + ^{1}_{0}n \longrightarrow ^{32}_{15}P + ^{1}_{1}H$$

The radio-P is separated from the sulfur by extraction with hot nitric acid. It is purified by repeated precipitation from ammoniacal solution using ferric hydroxide as a carrier. The iron is finally removed by extraction with ether. The purity of the P^{32} was also checked by a decay curve determination.

In both cases the elements in question were isolated without the addition of Rb or P carrier; therefore, the only Rb and P atoms in the solutions were those created by transmutation. The radioactive isotopes present were Rb^{86} and P^{32}. The activities of the solutions were 12 millicuries Rb^{86} per liter and 37 millicuries P^{32} per liter.

The number of radioactive atoms present is given by the expression

$$N = n\tau \qquad (1)$$

where n is the number of disintegrations per second and τ is the period of average life in seconds. τ is the reciprocal of the disintegration constant and can be expressed as $\frac{T\frac{1}{2}}{0.69}$, where $T\frac{1}{2}$ is the half-life in seconds. Making the above substitution in (1), one obtains

$$N = n\frac{T\frac{1}{2}}{0.69}. \qquad (2)$$

Since 1 millicurie is equal to 3.7×10^7 disintegrations per second, formula (2) may be modified as follows:

$$N = T\frac{1}{2}\frac{mc \times 3.7 \times 10^7}{0.69}. \qquad (3)$$

To express N in mols, it is only necessary to divide it by the number of atoms per mol, i.e., 6.06×10^{23} and (3) becomes

$$M = T\frac{1}{2}\frac{mc \times 3.7 \times 10^7}{0.69 \times 6.06 \times 10^{23}}.$$

For Rb^{86}, $T\frac{1}{2}$ = 19.5 days or 1.68×10^6 seconds; mc = 12, hence $M = 1.8 \times 10^{-9}$ molal.

For P^{32}, $T\frac{1}{2}$ = 14.3 days or 1.23×10^6 seconds; mc = 37, hence $M = 4 \times 10^{-9}$ molal.

Experiments were conducted with the above solutions of Rb* and P^*O_4 to determine the ability of plant roots to accumulate the ions under conditions of active metabolism. Low-salt barley roots from plants grown by the method of Hoagland and Broyer (1936) were used. The experimental material consisted of the "unbranched" roots arising from the seed. Their anatomy has been described by Jackson (1922). Before introducing the roots, each solution was adjusted to pH 5.5 with dilute KOH or HCl. The absorption-time curves were then determined as follows: a 2 cm. apical segment of barley root, from a three-weeks-old plant was placed in the stirred solution for a measured time interval. Following this, the segment was removed, washed thoroughly in running distilled water, and its activity determined by means of the Geiger-Müller counter. The segment was then again placed in the solution and the process was repeated until the desired time of exposure of the root to the solution was obtained. The experiment was not conducted over long periods in order to avoid possible injury as a result of excessive manipulation of the tissue. The solution was kept at 25°C., and at the conclusion of each experiment, the average diameter of the root was measured by means of a micrometer. The activities of the solutions were such that the counting periods were of short duration (one minute or less) and thus drying out of the tissue was inappreciable. The absorption curves are presented in figure 1. The activity of the solution indicated on the graphs corresponds to the activity of a volume of solution equal to the over-all volume of the root segment (i.e., tissue plus air spaces which in these roots may be considerable).

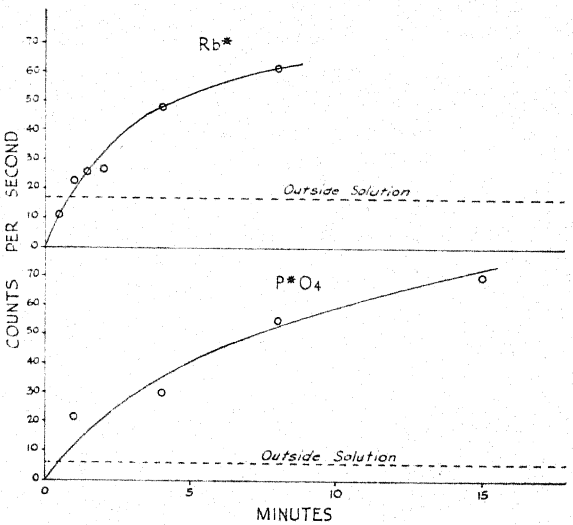


Fig. 1. Graphs showing the absorption of Rb^{86} and P^{32} by actively metabolizing apical root segments as a function of time. In each case the amount of radioelement in the segment is expressed in terms of counts per second. Root segments 2 cm. in length and about 0.4 mm. in diameter were repeatedly immersed in Rb*Cl and $KH_2P^*O_4$ solutions at 25°C. Following each immersion, the segments were washed in distilled water and counted. In each graph the dotted line denotes the activity of a volume of the bathing solution equal to the volume of the segment.

A set of experiments was carried out to determine the extent to which non-metabolic processes, such as exchange reactions, were operative in the absorption process. For this purpose the absorption-time experiments described above were repeated in all

details except that the culture solutions were maintained at 0°C. At this temperature the metabolic processes of the root had essentially ceased and the absorption measured was presumably non-metabolic in character. The non-metabolic absorption is described in curves A and B of figure 2. In these experiments it was considered desirable to determine the degree to which the absorbed radioelements could be released from the root by exchange for inert isotopes in the outside solution. Consequently,

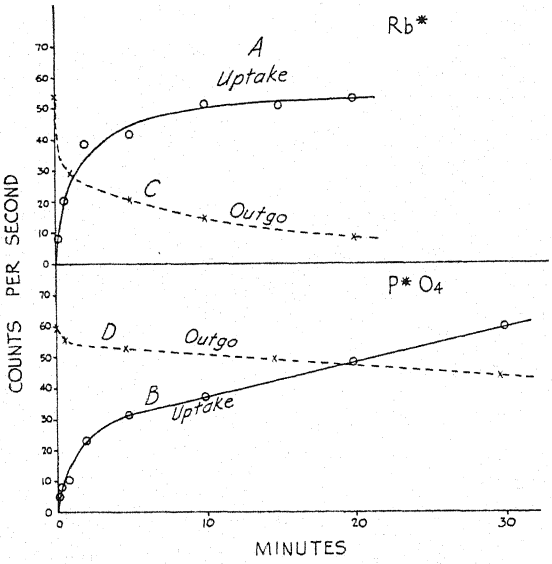


Fig. 2. Graphs showing the absorption at 0°C. of Rb^{86} and P^{32} by apical root segments and the subsequent release of the elements in exchange for inert isotopes. Root segments 2 cm. in length and about 0.4 mm. in diameter were repeatedly immersed in Rb*Cl and $KH_2P^*O_4$ solutions. Following each immersion the segments were washed in distilled water and counted (curves A and B). The segments were then transferred to 0.005 N solutions of RbCl and KH_2PO_4 and the process of successive immersions was repeated (curves C and D). The amount of radioelement in each segment is expressed in terms of counts per second.

at the conclusion of the absorption experiments the roots were placed in 0.005 N solutions of the inert elements (RbCl or KH_2PO_4) which were maintained at 0°C. The loss with time of the absorbed ions was determined by means of the counter as before. The release of the absorbed radioelements by exchange for inert isotopes in the culture medium is presented in curves C and D of figure 2.

A number of experiments were carried out to ascertain which regions of the root segment were most active in the non-metabolic absorption of the radioelements. Apical segments of barley roots one centimeter in length were placed in the radioactive solutions at 0°C. and allowed to remain for about 30 minutes. The roots were then removed and washed thoroughly in running distilled water. Following this, the average diameter of the segment was measured and the root was cut into sections 0.5 mm. and 1.0 mm. long. Since the sectioning was done by hand, it was not possible to cut sections smaller than 0.5 mm. without introducing a very considerable error. The activity of each section was then determined by means of the Geiger-Müller counter. Curves of activity per mm. length against distance from the root tip are given in figure 3. Each point on the curves of figure 3 represents the average of determinations on three very similar root segments. This method of presentation of the data was considered valid because of the great similarity in form of the individual curves even though the spread in concentration of comparable segments was in some instances as high as 30 per cent.

In the graphs of figures 1 to 3, amounts of the radioelements are expressed in terms of counts per second and counts per second per mm. of root. It should be emphasized that a given number of counts per second does not correspond to the same amount of element in all of the figures. For each experiment, the geometry of the counting arrangement was adjusted so that the activities measured would fall within the optimum counting range of the instrument.

Discussion.—It is clear from the curves of figure 1 that the root segments under conditions of active metabolism readily absorb Rb and PO_4 even when these ions are present in concentrations of 10^{-9} mols per liter. In the case of both ions an apparent accumulation was noted after about one minute, inasmuch as the concentration per unit volume of root was greater than the concentration per unit volume in the outside solution. The nature of this "accumulation" is as yet obscure. The possibility exists that it may constitute merely an exchange of radioactive isotopes in the solution for inert isotopes of the same element initially present in the root, or possibly for a different element. The degree to which metabolic processes were operative in the uptake was not determined.

In view of the complicated nature of ion-absorption in actively metabolizing root systems, it was decided to devote the major portion of this work to a study of non-metabolic absorption which in the view of many investigators constitutes the initial step in the uptake of ions. Inspection of the absorption curves for 0°C. (fig. 2, curves A and B) reveals a marked difference in the character of the non-metabolic uptake of Rb* and P^*O_4. The absorption of Rb* rapidly approaches a limiting value, whereas the absorption of P^*O_4 quickly assumes a constant rate. This difference in behavior is reflected in the corresponding outgo curves (fig. 2, curves C and D). Rb* is efficiently removed from the root system by exchange for inert Rb isotopes in the bathing solution, whereas P^*O_4 is, in comparison, but slowly removed at a nearly constant rate by exchange for ions containing the inert isotope.

The curves of figure 2 indicate that both Rb* and P^*O_4 when nonmetabolically absorbed are exchangeable for the inert isotopes. Also, P^*O_4 seems

to be more strongly bound in the root system than Rb*. However, considerable caution must be exercised in making generalizations from this single set of curves. In fact, preliminary experiments with roots grown under different conditions (barley seedlings cultured in the dark in $CaSO_4$ solutions) show a stronger binding of Rb* than that indicated in figure 2.

The graphs of figure 3 show clearly those regions in the root segment which are active in non-metabolic uptake of Rb* and P^*O_4. With both ions the major uptake occurs within a few millimeters of the root tip. In this region there is an "accumulation" of the ions over the outside solution. However, depending on the length of the segment this may or may not represent an accumulation for the segment as a whole.

The structure of the root cap is of such a nature that it would not be expected to be capable of pronounced ion-absorption. Possibly this accounts for the fact that the maxima in the absorption curves occur at about 0.75 mm. from the apex. Microscopic examination of a number of comparable barley roots showed an average length of the root-cap of 0.23 mm. According to the microscopic observations, the first 0.5 mm. section from the apex must have in each instance included the apical meristem as well as the root cap. Since the capacity for absorption of the root cap is unknown, no conclusions can be made regarding the ability of the meristem to absorb. All that can be stated is that in the first centimeter of the root, the maximum absorption occurs in the meristem or in a limited region immediately back of the meristem.

The root hairs were found to appear at an average distance of 5.2 mm. from the tip. The curves seem to indicate that the root hairs are not particularly active in the type of ion-absorption dealt with here.

The second maximum in the P^*O_4 curve (fig. 3) was noted in all three of the segments measured and is as yet unexplained.

As previously pointed out, the amounts of Rb and PO_4 involved in the curves of figures 1 to 3 are extremely small. For example, the amount of Rb corresponding to the maximum of curve A of figure 2 has been calculated to be 2×10^{-15} mols. By reason of the fact that radioelements without carrier are employed, amounts of this order can be measured with a considerable degree of accuracy by means of the Geiger-Müller counter and ion-absorption in individual roots can be investigated. However, because of the minute quantities involved in ion-absorption by individual roots, it is apparent that the presence of even small amounts of inert carrier will suffice to vitiate the method.

The general technique of sectioning individual barley roots in the study of ion-absorption and of metabolic activity has been employed by Prevot and Steward (1936), by Steward, Prevot and Harrison (1942) and by Machlis (1944). Since these investigators worked with actively metabolizing tissue, their findings are of limited assistance in the interpretation of ion-absorption at low temperature. An adequate explanation of the type of absorption shown in figures 2 and 3 must await further experimentation. However, it is of interest that both the Rb-cation and the PO_4-anion are non-metabolically absorbed and that the curve for PO_4 has none of the characteristics of an adsorption isotherm. Moreover, in unpublished experiments with I^- (I^{131} prepared without carrier) it has been found that this anion is readily absorbed at 0°C., but in a pattern

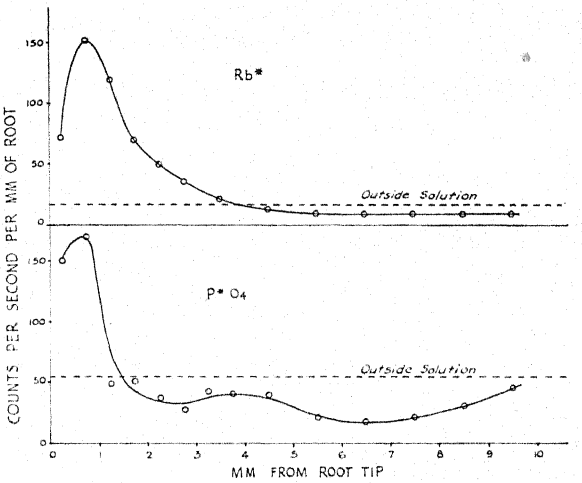


Fig. 3. Graphs showing the magnitude of non-metabolic absorption of Rb^{86} and P^{32} (expressed in counts per second per mm.) as a function of distance from the root apex. Apical segments of barley roots 1 cm. long, and approximately 0.4 mm. in diameter were immersed in Rb*Cl and $KH_2P^*O_4$ solutions for 30 minutes at 0°C. and subsequently sectioned and counted. The dotted lines labeled "outside solution" in each case corresponds to the activity of a volume of the bathing solution equal to that of 1 mm. of root segment.

dissimilar to both those of Rb and PO_4. Also it has been found that roots killed by treatment with ether or steam absorb I^{131} non-metabolically to a much smaller degree than living roots.

The idea that non-metabolic absorption is the initial step in normal salt accumulation forms the basis for a series of experiments now in progress. Absorption at 25°C. and the part played by translocation are being given particular attention. In addition, measurements employing the techniques presented here are being supplemented by radioautographs of the tissues.

SUMMARY

Actively metabolizing barley roots have been observed to accumulate radioactive Rb and PO_4 rapidly when present in solutions in concentrations of the order of 10^{-9} mols per liter (Rb^{86} and P^{32} prepared without inert carrier).

The non-metabolic uptake by barley roots of Rb and PO_4 in trace amounts has been studied. At

0°C. Rb* was absorbed by the roots in a form easily removed by exchange for inert isotopes in the bathing solution. P^*O_4 was absorbed non-metabolically in a form with difficulty removable by exchange for isotopes in the outside solution.

The non-metabolic uptake of trace amounts of Rb and PO_4 is to a major degree confined to the first few millimeters from the root tip. In this region the uptake represents an accumulation in that the concentration per unit volume of tissue is greater than in the bathing solution.

The application of the procedure used to the general problem of ion uptake is discussed.

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OBSERVATIONS ON CHYTRIDIACEOUS PARASITES OF PHANEROGAMS. I. PHYSODERMA MENYANTHIS DE BARY [1]

F. K. Sparrow

CHYTRIDIACEOUS FUNGI are predominantly aquatic organisms which reach their greatest development in fresh waters of the world. Here, as parasites of algae and microscopic animals and their eggs, and as saprophytes of algae and decaying organic debris, they contribute to the great cycle of disintegration so necessary to a balanced environment.

Certain of these fungi have, somehow, become adapted to existence within the living cell contents of certain terrestrial or semi-terrestrial phanerogams and have seemingly become obligate parasites on these hosts. The pathological effects induced by these chytrids are well known and familiar. Most commonly they manifest themselves in the formation of pronounced, often brightly colored warts, galls, spots, streaks or powdery pustules, composed for the most part of great numbers of the thick-walled, often dark-colored resting spores.

As early as 1833 Wallroth (1833) had described as *Physoderma* (*Urophlyctis*) *pulposa,* a chytrid found on members of the Chenapodiaceae. Schröter (1883) later studied the same species and recorded very briefly an interesting observation, namely, that in addition to the well-known endobiotic resting stage, visible in aggregate as pustules, warts, etc., there was also formed a superficial epibiotic zoosporangial stage, invisible save by microscopic observation. The vegetative system of this newly discovered thin-walled sporangial structure, in marked contrast to the extensive, endobiotic one on which the dark resting spores were formed, was short, bushy and distinctly monophagous. Similar, thin-walled sporangia were subsequently found by Büsgen (1887) in *Physoderma Butomi,* parasitic on *Butomus umbellatus* and in *Physoderma maculare* on *Alisma Plantago-aquatica* by Clinton (1902). Büsgen noted that upon the germination of the thick-walled resting spores zoospores were produced which gave rise, apparently without copulation, either to the strongly polycentric endobiotic phase which bore the resting spores, or to so-called "ephemeral sporangia." These sporangia were essentially like those described earlier by Schröter in *P. pulposa.* They liberated at maturity zoospores which were like those produced by the thick-walled rest-

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ing spores save that the oil globule was somewhat smaller. After discharge the sporangia exhibited an unusual capacity, at least among chytrids, for internal proliferation, and a second and sometimes a third sporangium were successively formed. The zoospores, so far as Büsgen could determine, produced only new ephemeral sporangia and he concluded that these thin-walled reproductive structures were simply a means of dispersal of the fungus.

Clinton's subsequent observations on the parasite of *Alisma* confirmed beyond question the regular occurrence in *Physoderma* of these two contrasting epibiotic, monocentric, and endobiotic, polycentric phases. He was inclined to believe that the epibiotic stage was formed primarily on the aquatic, and the endobiotic on the aerial leaves of *Alisma* although, like Büsgen, he found instances in which the two occurred side by side. Clinton suggested that the endobiotic phase may have developed from zoospores which after settling down on the host leaf had become exposed to air, whereas the epibiotic or "ephemeral" sporangia developed from zoospores which had remained submerged at all times during their development. One feature is apparent from Clinton's observations to which no reference is made, namely, that in the sequence of development of the fungus, the epibiotic phase appears to precede the endobiotic.

The object of the present paper is to give an account of a series of observations initiated primarily to determine whether or not this little-known monocentric stage is present in *Physoderma Menyanthis* in which it has not been reported and, if possible, to determine the significance of this stage in the life history.

Through the kindness of Prof. F. T. Brooks of the Botany School, Cambridge University, it was possible to obtain living material of the fungus. The writer wishes to express his thanks to Professor Brooks for this material.

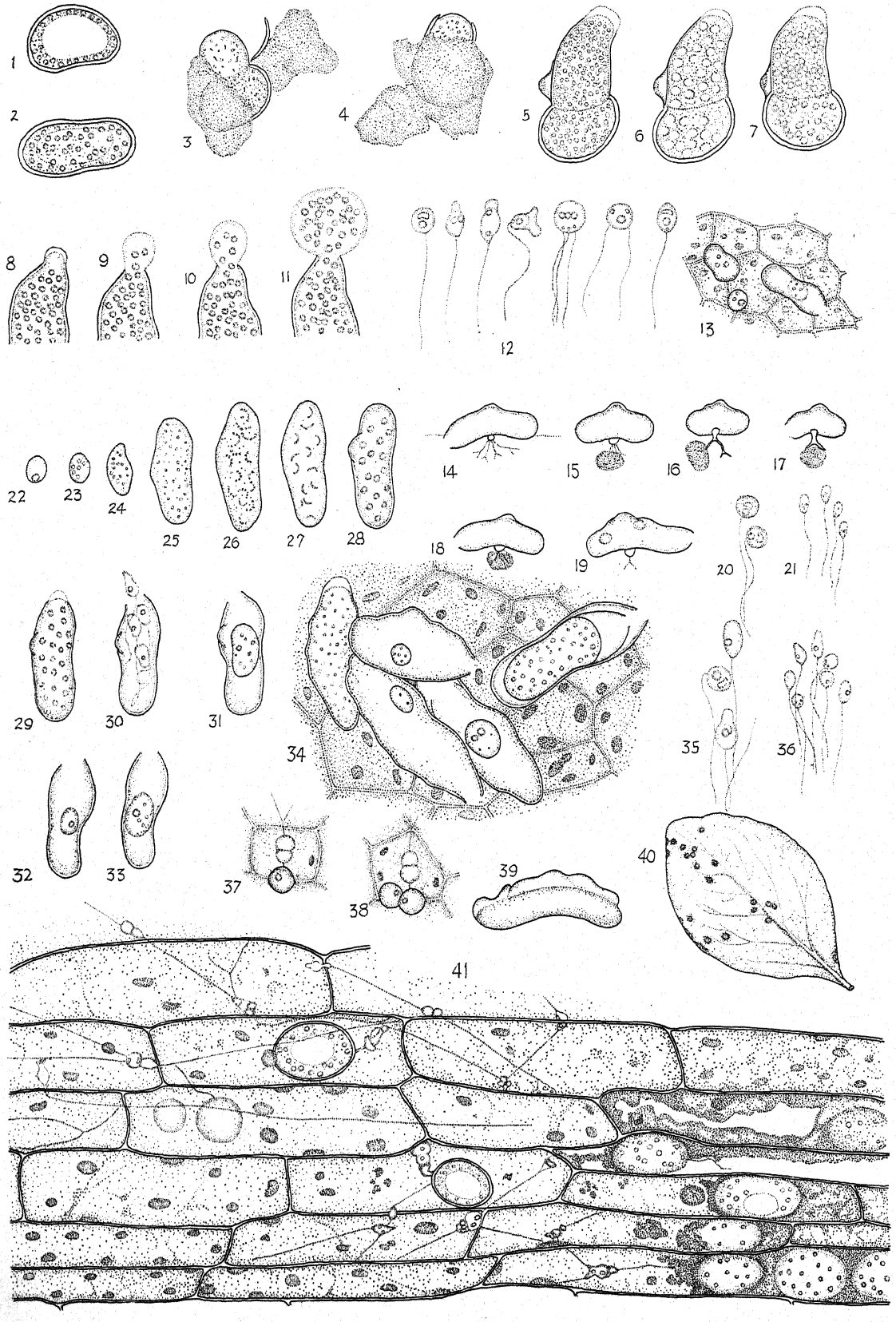
Materials and methods.—The material of *Physoderma Menyanthis* was obtained from a stand of *Menyanthes trifoliata* growing in a damp, boggy swale bordering a prehistoric dike in the vicinity of the River Lark near Mildenhall, Suffolk, England. Abundant infection had been noted at this site during the summer of 1931 by Dr. W. J. Dowson of the Botany School, and a similar infection was found the following year by Professor Brooks and the writer. At the time of collection in October the host plants were already dead and in some cases partly disintegrated. The resting spores of the fungus were unquestionably fully mature. The fungus has subsequently been found in fair abundance in several places in Michigan.

Since the germination of the resting spores was the obvious point at which to initiate the proposed study, and since it was found that they would not germinate immediately after collection, it was necessary to place the spores under conditions that would favor this process. From their natural habitat it was presumed that if the resting spores were kept moist and subjected to prevailing winter temperature conditions, they should germinate after their natural "after-ripening" period. Accordingly, dead infected leaves were placed in cheese-cloth bags and stored in perforated tin boxes between layers of wet sphagnum and dead *Menyanthes* leaves obtained from the place of collection. These boxes were left outside on the roof of the Botany School throughout the mild English winter, water being added from time to time to keep the contents moist. Periodic germination tests indicated that by mid-December the resting spores were capable of germination and from then on until the supply was exhausted in March, excellent material for germination was available.

In preparing the resting spores for germination the following methods were employed. Small clumps of debris consisting of dead host tissue and resting spores were teased apart in thin films of water on cover glasses. When considerable separation had been achieved and larger bits of host debris removed, the cover glasses were ready for use in hanging drop preparations. Open watch glasses containing dissected clumps of spores in a relatively deep layer of distilled water were also used but gave inferior results.

Resting spores of *Physoderma Menyanthis* were exceedingly slow in germinating when taken directly from the host tissue, placed in hanging drop cultures and left at laboratory temperature (17°C.), approximately seven days being necessary. However, it was found that those stored for two weeks at near freezing temperature in an ice box could then be induced to germinate within two days after removal. Since seeds of *Menyanthes trifoliata* were not available, in order to obtain host material it was necessary to force plants from underground rootstalks.[2] Several methods were used for inducing infection. In the first, drops of water containing zoospores from germinated resting spores were placed by means of a brush on undisturbed young leaves of the host and the whole plant covered with a large bell jar in order to prevent rapid evaporation. Those portions of the leaf beneath the drop were subsequently removed and examined for sporangia. Parts of undisturbed leaves were also allowed to dip into small dishes of water containing germinating resting spores. Another, and the most useful method for observing the actual infection and development of the ephemeral sporangia was the following. Small chips of living leaf or stem tissue were mounted on a cover glass in a thin film of water containing germinating resting spores, care being taken that the epidermal surface of the host material was next to the surface of the glass. The preparations were then inverted and set up in van Tieghem cells in the usual

[2] It is a pleasure to acknowledge my indebtedness to Mr. Humphrey Gilbert Carter, Keeper of the University Botanic Garden, Cambridge, for supplying rootstalks of *Menyanthes*.


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manner in diffuse daylight at room temperature. Under these conditions, the bulk of the host tissue remained alive for approximately a week, a time sufficient for the observations. Using this set-up, the activities of the zoospores and their subsequent fate could be followed directly and with relative ease.

PHYSODERMA MENYANTHES.—Descriptions of the resting stage of this species and of the lesions caused by it on the host plant have been published by a number of investigators, including deBary (1864), Lüdi (1901), Büsgen (1887), and Clinton (1902).

Infected leaflets of *Menyanthes* bearing the resting spores show, irregularly distributed, slightly raised circular areas which vary from pin point size to 1.5 mm. in diameter (fig. 40). Although distinct and rounded in outline on the lamina of the leaflet, when occurring on the margin and petiole, they may become confluent and somewhat elongate. There is no splitting or shredding of the adjacent host tissue, which in most cases appears to undergo nearly normal growth. When young the spots are somewhat pinkish but at maturity assume a dark chocolate color.

The resting spores are aggregated within the pustulate area where they occur in dense groups, linear series or occasionally separate. Usually they are fused with dark brown, disintegrated host material, so much so that it is difficult to obtain single resting spores. No trace remains of the vegetative system on which they were formed. A striking feature of the spores especially when compared, for example, with those of *P. Zeae-maydis,* was their irregularity in shape. In general they were somewhat elongate ovoid and slightly flattened on one face (fig. 1, 2). Their precise shape was usually obscured by their tendency to form together with host tissue dense aggregations. The wall is pale to chestnut brown, smooth and relatively thin. The contents bear a large central oil globule surrounded by finely granular protoplasm within which are numerous minute hyaline globules. If pits are present at all, as stated by Jones and Drechsler (1920), they must be exceedingly minute for none was demonstrated after treatment with the usual reagents.

GERMINATION OF THE RESTING SPORE.—The first indication of germination is the beginning of fragmentation of the large oil globule. As this successively separates into smaller and smaller globules, the protoplasm becomes more distinctly granular and homogeneous and bears the minute, refractive, somewhat angular remains of the globule. The wall of the resting spore then splits, probably circumcissally and there is formed a slight hyaline protrusion (fig. 3, 4). Due to the presence of large amounts of residual host material around the resting spore, it is not usually possible to detect with certainty the precise method of dehiscence. In occasional instances it appears that a cap is thrown back, but often no such well-defined structure can be observed, a fact also noted by Clinton (1902) in this species.

During the ten to thirty hours following the opening of the resting spore, there is very slowly formed a stubby, finger-like, often somewhat curved, thin-walled, hyaline sac usually termed a sporangium. As this structure slowly elongates, the more or less evenly distributed refractive granules making up its contents coalesce to form the highly refractive globules of the individual zoospores (fig. 5–7). Meanwhile, at the somewhat blunt apex of the protrusion there appears, almost coincident with the completely homogeneous granular stage of the contents, an arc-like zone of clear protoplasm. This increases in thickness and forms the conspicuous discharge papilla. At maturity this is differentiated into a distal region of very high refractivity from which may occasionally be observed emerging a downwardly-directed central peg, and a less refractive region bearing transient vacuoles which is bounded proximally by the granular cytoplasm. During maturation the walls of the sporangium become sharper in outline, apparently thickening, and the conspicuous globules assume a regular arrangement in the contents. There is not, however, any visible evidence of the cleavage of the cytoplasm into zoospores. At the

Fig 1–41.—*Physoderma Menyanthis.* Fig. 1–21, × 800; fig. 22–39, × 1100; fig. 40, natural size; fig. 41, × 800.—Fig. 1. Resting spore in optical section.—Fig. 2. Another, in surface view.—Fig. 3–4. Early stages in germination of the resting spore.—Fig. 5–7. Stages in the aggregation of the fatty materials to form the globules of the zoospores. An operculum is visible.—Fig. 8–11. Initial stages in the discharge of the zoospores.—Fig. 12. The four to the left, various shapes assumed by the normal zoospores. The next two, abnormal incompletely cleaved zoospores. Right hand one, ellipsoidal normal zoospore showing globule, nuclear cap and basal structure near posterior flagellum.—Fig. 13. Various stages of development of the ephemeral sporangia on the surface of a bit of *Menyanthes* tissue.—Fig. 14–19. Discharged ephemeral sporangia showing various shapes.—Fig. 20. Zoospores from germinated resting spores.—Fig. 21. Zoospores from ephemeral sporangia (same magnification).—Fig. 22–28. Successive stages in the development of an ephemeral sporangium.—Fig. 29. Mature ephemeral sporangium.—Fig. 30. The same discharging its zoospores. The lowermost structure will give rise to a new sporangium and is not a zoospore.—Fig. 31. Same sporangium showing at a later time the developing secondary sporangium.—Fig. 32–33. Similar to the preceding.—Fig. 34. Group of ephemeral sporangia on surface of host tissue. The left one is mature, the three central ones have discharged their first generation of zoospores and the secondary sporangia are developing. The right hand one is producing a tertiary sporangium, two others having previously emptied their contents.—Fig. 35. Zoospores from resting spores.—Fig. 36. Zoospores from ephemeral sporangia for comparison (same magnification).—Fig. 37. Initial stage in formation of the endobiotic, polycentric system; the first turbinate organ has been formed.—Fig. 38. Two encysted zoospores, one of which has formed the first turbinate organ.—Fig. 39. Unusually gibbose ephemeral sporangium.—Fig. 40. Pustules formed by the endobiotic phase on leaflet of *Menyanthes.*—Fig. 41. Portion of the leaf tissue of *Menyanthes* artificially inoculated with the fungus showing the polycentric endobiotic system, and resting spores. The turbinate cells are irregular in shape and so filled with strongly refractive material as to obscure their true shape.

moment of discharge, the double contour of the papilla is lost, the refractive material suddenly expands and the zoospores emerge (fig. 8 to 11). These are swept out passively and rapidly and form a globular motionless mass imbedded in the material of the discharge papilla. When a third of them or, in some instances, nearly all have emerged, the material causing them to aggregate appears to become dissipated in the medium. They then fall apart into a loose group for an instant before darting away and assuming individual motility. Under less favorable environmental circumstances, nearly all of the zoospores escape individually from within the germ sporangium by flagellar action.

The zoospore thus produced is typically chytridiaceous (fig. 12), somewhat ovoid, 8–9 μ long by 5 μ in diameter, with a conspicuous, eccentric, protruding colorless refractive globule and a long posterior flagellum. In both living and stained material the characteristic nuclear cap is unusually conspicuous. Under poor environmental conditions abnormal bi-, tri- and multi-flagellate zoospores are commonly produced (fig. 12). Movement of the normal spores may be characterized as an even swimming interspersed with occasional hops and sudden stops during which amoeboid motion may be assumed.

After a variable period of swarming of not over 24 hours duration, the length doubtless dependent upon environmental conditions prevailing in the medium, the spores come to rest in the water, lose their flagella and encyst. If they are not in contact with living tissue of the host, usually no further development occurs. If, however, they settle down on the outer surface of the epidermis of the host, the formation of the so-called "ephemeral sporangia" ensues.

DEVELOPMENT AND STRUCTURE OF THE EPHEMERAL SPORANGIA.—A series of van Tieghem cells was set up as previously described and maintained at approximately 15.5°C. Bits of the outer tissue of the winter bud of *Menyanthes* were placed in the water film swarming with recently liberated zoospores from germinated resting spores. A control series was also prepared, the bits of host tissue being suspended in sterile water alone.

Typically, after 24 hours the zoospores from germinated resting spores had come to rest (fig. 22). Little change was noticed during the ensuing 24 hours in those resting on the outer host surface. The succeeding 24 hours, however, witnessed the initiation of development of the young monocentric thalli. The first visible change in the contents of the encysted zoospore is the fragmentation of the oil globule (fig. 23). Almost coincidently, there is initiated a gradual enlargement and elongation—primarily in a plane parallel with host surface—of the spore body on the ventral or "belly" face (fig. 24). A dorsal portion of the spore body, however, apparently remains rigid and persists as a more or less conspicuous hump or papilla (fig. 14 to 19, 30), a type of development recalling that found in sporangia of *Chytridium Schenkii* and related species, and noted previously by Büsgen in *P. Butomi*. Owing to the opacity of the host, early stages in the development of the rhizoidal system could not be followed. It is reasonable to suppose, however, that the usual penetration tube is produced which bores through the wall and contacts the living host contents, and from this the small apophysis and short bushy, rhizoidal system, structures easily observed on developing sporangia (fig. 14 to 17), are formed.

The sequence of protoplasmic changes undergone by developing ephemeral sporangia is essentially like that observed in the sporangium formed at the germination of the resting spore, the most noticeable feature being the organization of the globules of the zoospores (fig. 22 to 28).

At maturity the colorless, ephemeral sporangium bears a prominent discharge papilla and in dorsal view is somewhat irregularly slipper-shaped in outline. Laterally, it is pronouncedly gibbose (fig. 14 to 19, 39), the persistent unexpanded portion of the original zoospore case being a conspicuous feature. It measures 24–52 μ in length by 8 μ in greatest width, the majority being 20–40 $\mu \times$ 8–10 μ.

The zoospores, of which there are usually 20 to 30, escape upon the deliquescence of the papilla. As in the discharge of zoospores from germinated resting spores, the first liberated swarmers are imbedded in the hyaline material of the papilla and move passively from the sporangium. Later ones, however, usually escape in amoeboid fashion by their own efforts (fig. 30). They are somewhat ellipsoidal and are provided at the time of emergence with a single posterior flagellum 15–18 μ in length. They differ from the zoospores produced by the germinated resting spores primarily in size. Whereas those formed at the germination of the resting spores are 8–9 μ long by 5 μ in diameter, those produced by the ephemeral sporangia are 5–7 $\mu \times$ 3–5 μ. The flagella are proportionally shorter in the smaller spores. Highly characteristic features of the larger zoospore are the conspicuous, eccentric oil globule and dull gleaming nuclear cap. Zoospores from ephemeral sporangia, however, bear a minute refractive globule, and the nuclear cap although probably present is not evident. In their movement they resemble the larger zoospores, alternately darting evenly and swiftly through the water and suddenly crawling in amoeboid fashion over the substratum.

After discharge there is often observed within the sporangium at the point of its attachment to the rhizoidal system a structure resembling a quiescent zoospore (fig. 13, 32). After approximately 24 hours this body has doubled in size (fig. 31, 33 to 34) and in another 24 hours has become a mature sporangium. Zoospores similar in size and shape are eventually discharged and a third reproductive structure may be formed (fig. 34). Such sporangial proliferation has also been noted by Büsgen (l.c.) in *P. Butomi* and by Clinton (l.c.) in *P. maculare*, where as many as 5 to 6 "nested" sporangia may be formed.

Clinton declared that these secondary sporangia arose by successive swelling of the apex of the septum separating the rhizoidal system from the sporangium. The present observations seem, rather, to point to the necessarily nucleated rudiment being laid down at the time of formation of the zoospores, although further observations will be necessary before this point can be settled with certainty.

FATE OF THE ZOOSPORES FROM EPHEMERAL SPORANGIA.—Both Büsgen and Clinton ascertained that the zoospores formed by ephemeral sporangia produce new sporangia of the same type. To obtain information on this point in *Physoderma Menyanthis,* a series of van Tieghem cells was prepared as before with zoospores from germinated resting spores and bits of living host tissue. Suitable controls were also set up. After three days, all the swarmers had come to rest and ephemeral undischarged sporangia were present in abundance. The bits of host tissue were then removed from the van Tieghem cells and washed in several changes of sterile water to remove any swarmers which might possibly have persisted. They were then placed in new van Tieghem cells in sterile water together with fresh bits of host tissue especially notched for future identification. After three to four days the notched bits of tissue bore abundant ephemeral sporangia which must necessarily have been formed from zoospores discharged from the ephemeral sporangia on the original bits of *Menyanthes* tissue. It can be seen, therefore, that this phase of the fungus can be multiplied not only by the zoospores of the initially formed sporangium but by zoospores from secondarily produced sporangia formed by internal proliferation and by zoospores from new ephemeral sporangia. Furthermore, according to these observations, two types of swarmers from two different structures form similar sporangia, namely, the zoospores from germinated resting spores and those from ephemeral sporangia (fig. 20, 21, 35, 36).

Because of the opaque nature of the host tissue, little could be observed of the effect of the ephemeral thalli on the host cells. Although some disintegration of the chloroplasts was occasionally seen, it was evident that, unlike the endobiotic stage, no hypertrophy, discoloration, etc., was produced. Such effects as were found seemed confined to the particular host cell involved. Under more favorable conditions of observation, such, for example, as those afforded by young seedlings, it is hoped that further, more concise information can be obtained on this point.

One of the primary objectives of this study was to determine whether zoospores from ephemeral sporangia were not, in fact, gametes, which fused to form a zygote which in turn established the strongly polycentric endobiotic system. It seemed more likely that these swarmers, rather than those from the resting spores, would be the ones to function in this manner. Occasional cases of zoospores with two flagella, such as noted by Ojerholm (1934) in *P. Zeae-maydis,* were seen in both types of swarmers studied (fig. 12), but they were never observed in the act of fusing and must be regarded as abnormal, "giant" spores occasionally formed in the sporangia of most aquatic Phycomycetes. If such a fusion does occur—and further investigations may yet substantiate it—unfavorable environmental conditions or possible incompatibility of the swarmers may have nearly or completely prevented it under the conditions of observation.

THE ENDOBIOTIC PHASE.—At approximately the time the first formed ephemeral sporangia had discharged their zoospores and secondary sporangia were being formed, the initial stages in the formation of the endobiotic, polycentric phase were observed. Several instances were noted in which a zoospore cyst, approximately 4–5 μ in diameter, somewhat larger than that formed by the quiescent zoospore from the ephemeral sporangium, was resting on the outer surface of the host. Directly beneath it there was faintly visible a two-celled turbinate organ or "Sammelzellen" connected to it by a delicate rhizoid (fig. 37, 38). Distally, this turbinate structure, so characteristic a feature of the mature polycentric rhizoidal system, gave rise to a branched rhizoid, as yet of limited extent. Since the host tissue rarely remained viable for more than a week, further studies on the extension of this thallus throughout the host cells was impossible. Enough was observed, however, to determine that, in contradistinction to the ephemeral thallus, this one was definitely polycentric (fig. 37, 38).

Since the fate of individual swimming spores, of which there were many in each van Tieghem cell, could hardly be followed, it was impossible to ascertain the origin of the epibiotic cysts from which the polycentric, endobiotic rhizoidal system had been derived. These cysts are definitely larger than those of individual zoospores from ephemeral sporangia (4–5 μ in diameter as compared with 2.5–3 μ) and in this respect are similar to the cysts of zoospores from germinated resting spores (5 μ). However, the large epibiotic cysts were always found four to five days after setting up the van Tieghem cells, at which time the vast majority of those zoospores from germinating resting spores had either disintegrated, or if resting on host tissue, had produced ephemeral sporangia which had already discharged their first "generation" of zoospores. If these cysts were indeed from the zoospores produced at germination of the resting spores, then the rate of development of the endobiotic phase must be exceedingly slow.

Since further stages in the life history of the parasite could not be studied by the van Tieghem cell method, it seemed nonetheless of interest to carry the cycle to completion, even if methods less susceptible to direct observation would have to be employed.

Menyanthes plants were forced in the greenhouse, the pots standing in water under large bell jars to insure a high humidity. Since zoospores from ephe-

meral sporangia could not be obtained in sufficient quantity to be utilized as an inoculum, resting spores were germinated and the active zoospores painted on parts of the leaves and stems with a camel's hair brush. Other methods of application were tried but this one seemed the most successful. Both upper and lower surfaces were treated and the areas where the inoculum was applied were circumscribed with a line in India ink. It was found very difficult to keep the liquid bearing the zoospores in place because of the strongly cutinized nature of the surface. In four of twelve areas thus treated, there appeared after four weeks, pale, pink, then reddish-brown pustules primarily on the upper surface of the host leaf. Young areas when removed and teased apart showed the typical polycentric rhizoidal system bearing numerous highly refractive turbinate cells and the typical resting spores in various stages of maturity (fig. 41).

Further work on this stage of the fungus was not feasible at the time and is reserved for a future study.

SUMMARY

The present paper describes certain phases in the life history of a chytrid parasite of a bog-inhabiting phanerogam, *Menyanthes trifoliata*. The fungus, *Physoderma Menyanthis*, winters over in the resting spore stage. At germination of the resting spore, it cracks open and a thin-walled, finger-like structure is produced within which are formed zoospores. These swarmers may eventually settle down on the surface of living host tissue, penetrate it, and form a monophagous, bushy rhizoidal system within. The epibiotic body of the spore steadily enlarges and forms at maturity a thin-walled, so-called "ephemeral" sporangium. This forms zoospores which are somewhat smaller than those formed at the germination of the resting spores. These zoospores apparently form only new ephemeral sporangia. New ephemeral sporangia may also be formed by internal proliferation of discharged ones.

About five days after the swarming of the zoospores from germinated resting spores, the first evidences of the thallus on which will be borne the dark resting spores is observed. Contrasted with the epibiotic thallus this one is polycentric, bearing numerous turbinate organs and produces visible hypertrophy of the host.

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ISOLATION OF 3–INDOLEACETIC ACID FROM IMMATURE CORN KERNELS [1]

A. J. Haagen-Smit, W. B. Dandliker, S. H. Wittwer, and A. E. Murneek

THE PERIODIC presence of natural auxin-like substances within the reproductive parts of many higher plants might conceivably provide a key to the experimental elucidation of problems concerned with seed and fruit formation and development, the physiology of which is little understood (Murneek, 1939). A powerful stimulus to plant growth following pollination and subsequent fertilization in the tomato has been demonstrated by Murneek (1926). That the occurrence of hormones in reproductive tissues of corn is associated with the critical developmental processes of gametophyte and seed formation has been suggested by Wittwer (1943). These periods of hormone production, which seem to follow the union of chromosomes in the synaptic reaction and of the fusion of nuclei in the syngamic process, are accompanied by parallel or simultaneous growth phenomena in the corn plant which give rise to two maxima in the growth curve as outlined by Briggs, Kidd and West (1920). Consequently, our interest has been focused on the nature of active hormone substances so abundantly present in immature seeds, perhaps causal for the overall acceleration in plant growth.

3-indoleacetic acid has been isolated from the auxin liberated by the alkaline hydrolysis of corn meal and dormant corn kernels by Haagen-Smit *et al.* (1941) and Berger *et al.* (1944), respectively. The auxin liberated in this way constitutes the so-called "bound" or "precursor" auxin. The fraction which occurs in a free and biologically active form after mere extraction with water or organic solvents has not been previously isolated from either dormant or immature corn.

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Both Avery *et al.* (1942) and Wittwer (1943) have studied the changes in auxin concentration during the development of young corn kernels. The results of these workers indicate a maximum in the free auxin concentration at about 10 to 15 days after fertilization. This auxin is of special interest because it occurs in a readily soluble and active form in the fresh tissue, at the time when growth activity reaches a peak in the entire corn plant organism.

The following describes a procedure by which 3-indoleacetic acid was isolated from the free auxin fraction of immature kernels.

One hundred kg. of fresh corn kernels[2] in the milk stage, that is about fifteen days after fertilization, was extracted with 95 per cent ethanol at room temperature. The resulting water-alcohol solution was concentrated under reduced pressure to 3,700 cc. of thick sirup. This concentrate contained an amount of free auxin equivalent to 1.48 gm. of 3-indoleacetic acid. The standard *Avena* test was used as a bioassay throughout. The once-extracted corn kernels contained an amount of auxin equivalent to 1.20 gm. of 3-indoleacetic acid, as determined by maceration and extraction with water at room temperature.

The sirupy concentrate was acidified to pH 3 and extracted with diethyl ether; the acid fraction was removed from the ether by extraction with aqueous sodium bicarbonate solution. The bicarbonate solution was acidified and the free acids removed by re-extraction with ether. This ether solution was evaporated under reduced pressure and yielded 10.5 gm. of a crude acid fraction containing an amount of auxin equivalent to 1.09 gm. of 3-indoleacetic acid, as shown by maceration and extraction with water for two hours at 25°C.

Both the auxin in this crude acid fraction and the free auxin remaining in the extracted corn kernels were stable toward hot alkali and labile toward hot acid. Treatment with 5 per cent HCl for 3.5 hours at 98°C. resulted in about 86 per cent destruction while no measurable destruction occurred in 1.0 N potassium hydroxide under the same conditions. Synthetic 3-indoleacetic acid underwent 40 to 50 per cent destruction with the above acid treatment, and none by heating with alkali.

Approximately half (5.02 gm.) of the above crude fraction containing the equivalent of 0.52 gm. of 3-indoleacetic acid was subfractionated by dissolving it in 60 per cent ethanol and extracting several times with benzene. The benzene solution was extracted with 50 per cent methanol and then water. The methanol and water extracts were combined and partially evaporated to remove most of the methanol. The aqueous residue was extracted with ether and the ether solution evaporated *in vacuo*. The 1.25 gm. of residue which remained contained the equivalent of 0.26 gm. of 3-indoleacetic acid.

Of this fraction, 0.87 gm. was extracted with hot petroleum ether and the insoluble portion extracted with hot xylene. The remaining residue was inactive in the *Avena* test. The xylene solution was evaporated under reduced pressure and the residue (505 mg.) extracted with hot cyclohexane. The cyclohexane solution on cooling yielded crude crystalline 3-indoleacetic acid. The material insoluble in cyclohexane was again extracted with hot xylene and only a very small inactive residue remained. The xylene solution was again evaporated yielding 328 mg. of residue which was found to contain the equivalent of 69 mg. of 3-indoleacetic acid.

This fraction was treated with lead ion in dilute acetic acid. A precipitate formed which, after removal of the lead ion, weighed 123 mg. The filtrate from the lead precipitation gave a residue of 118 mg., which, on cooling in chloroform, yielded 24 mg. of crude crystalline 3-indoleacetic acid. The material precipitated in the first treatment with lead ion was treated again the same way. The filtrate this time yielded 27 mg. of crude 3-indoleacetic acid directly. This no doubt had been adsorbed on the first precipitate.

The crude crystalline fractions obtained from the cyclohexane extractions and the two lead precipitations were combined and recrystallized from chloroform. From this, 49 mg. of white, thin, flat plates were obtained. A second recrystallization from chloroform yielded 42 mg. of a compound which melted at 165.7 to 166.5°C. (all melting points are uncorrected) while an authentic sample of 3-indoleacetic acid melted at 165.5 to 166.5°C. An intimate mixture of the two showed no melting point depression.

The following percentage composition was found for the crystalline material from corn:

	Calculated for $C_{10}H_9O_2N$	Found
Carbon	68.54%	68.99%
Hydrogen	5.18%	5.17%
Nitrogen	8.00%	8.06%

The material from corn was found to have the same activity in the *Avena* test as synthetic 3-indoleacetic acid.

Discussion and conclusions.—From the foregoing data, it is concluded that the free auxin detectable by the *Avena* bioassay method in immature corn is chiefly or perhaps totally 3-indoleacetic acid. This assumption is at least partially justified by the fact that the auxin in the crude ethanol extract and in the extracted corn kernels, like 3-indoleacetic acid, is readily destroyed by hot acid and is stable to hot alkali under the conditions stated above. The yield of pure crystalline material based on the auxin content of the crude alcoholic extract is about 9 per cent, after making allowance for the fact that only a part of each active fraction was used for further purification.

[2] A single cross (R136 × F6) of two self-fertilized lines of yellow field corn was the material used.

The formation of heteroauxin following syngamy in the developing seed suggests a possible role played by this substance in formation of embryos, fruit setting and their development. However, there is still the possibility of the presence of other hormone materials more specific for fruit setting. Such components, if they exist, may not necessarily be active in the *Avena* assay, or be released in biologically active forms by the solvents employed. The mere creation of 3-indoleacetic acid, which is relatively ineffective in fruit setting, can hardly account for the remarkable growth stimulatory effects and the set of fruit which generally accompanies pollination and subsequent fertilization in many higher plants.

SUMMARY

On the basis of acid and base destruction tests the free auxin extractable with 95 per cent ethyl alcohol from immature corn kernels and detectable by the *Avena* test, consists chiefly of 3-indoleacetic acid. The total amount is calculated to be 2.68×10^{-5} gms. per 1 gm. of kernel. Nine per cent of this amount was isolated in crystalline form and characterized as 3-indoleacetic acid.

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RELATION OF ENVIRONMENTAL AND HEREDITARY FACTORS TO ASCORBIC ACID IN CABBAGE [1]

Frederick G. Smith and J. C. Walker

Ascorbic acid content is now recognized as a quality of major importance in many plant foods. Cabbage is one of the most economical natural sources of this vitamin in the American diet. The possibility of increasing ascorbic acid content by control of certain environmental factors and by breeding has received attention in the case of this crop (Burrell *et al.*, 1940; Murphy, 1942; Poole *et al.*, 1944). The wide range in this vitamin reported for cabbage varieties under various conditions (Gould *et al.*, 1936; Floyd and Fraps, 1939; Burrell *et al.*, 1940; Murphy, 1942; Poole *et al.*, 1944) points to the complexity of the factors involved.

Adequate studies of ascorbic acid variation in crop plants depend upon effectual control of sampling errors and upon an analytic method which is simple in execution yet sufficiently specific and quantitative. Insufficient attention to these may account for some of the wide variation reported.

Oxidation of ascorbic acid with 2,6-dichlorobenzenoneindophenol is by far the most carefully studied and widely used basic analytic method. The specificity of this method and the estimation of bound ascorbic acid and of dehydroascorbic acid have been discussed repeatedly. The consensus of opinion seems to be that with proper handling extraction is substantially complete (Mindlin and Butler, 1937; Mack and Tressler, 1937; Reedman and McHenry, 1938; King, 1941; Harris and Olliver, 1942; Anon., 1944). Furthermore, repeated comparisons of certain of the modifications of the method with animal assay have shown good agreement (Harris and Olliver, 1942; Dunker *et al.*, 1942). Numerous technical modifications have been described, but the application of the photoelectric colorimeter by Mindlin and Butler (1937) and Bessey (1938) and the replacement of hand grinding in extraction by the mechanical blendor by Davis (1941) and Morell (1941) have done most to make feasible the analysis of large numbers of samples of plant material.

Experimental results.—*Analytical methods.*—In adapting the method to the present program the several steps and conditions of Morell's technic were first re-examined. It was found at once that variation within the cabbage head increases the problem of sampling. Table 1 illustrates typical variation within the head. After trying several methods, the following one was chosen as the best compromise

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TABLE 1. *Typical variation in ascorbic acid content of various samples from marketable portion of cabbage head.*

Variety	Lot number	Mg per 100 g fresh tissue in Core	Midrib	Leaves	Basal slice	Middle slice	Top slice
All Head Early.....	1	60.0	50.7	56.4	...	...	...
	2	70.3	64.5	60.1	...	...	...
Globe	1	52.5	41.8	32.8	41.8	38.1	37.2
	2	...	...	...	40.6	38.7	37.3
	3	...	...	...	38.4	...	31.8
All Seasons	1	...	...	...	56.5	...	51.8
Danish Ballhead	1	...	...	...	49.0	...	42.7
Wisconsin Ballhead...	1	84.0	59.5	49.4	...	...	...
	2	82.5	64.4	46.2	...	...	...
	3	66.4	47.6	42.4	...	...	...
Bugner	1	81.5	68.5	50.7	40.4	...	36.5
	2	...	...	...	40.4	...	34.8
	3	...	...	...	64.1	54.0	49.0

between economy and accuracy for the present purpose. The head was quartered along its axis and three representative 15-g slices were cut from a single quarter perpendicular to the axis, the first near the base including some of the core, the second through the center usually at the tip of the core, and the third through the leafy material above the core. These were routinely lumped together for extraction, giving one analysis per head. About half the heads of several varieties examined showed significant variation among these slices, and it was concluded that this method was more accurate than a single slice per head, especially when relatively small numbers of heads in a sample were used. Furthermore, it was necessary to use as large slices as possible to minimize the effect of variation in ascorbic acid in the different parts of the individual leaves composing the head. Three 15-g slices were found to be as large an amount of tissue as could be efficiently extracted in the blendor without using more than 200 ml of extractant and a longer time of extraction than two minutes. With less than 15 g it was increasingly difficult to cut uniform slices especially from large heads. The sampling of a single quarter was justified by the close agreement found between opposite quarters. ($F = 0.39$ for analysis of variance of a series of determinations).

The adequacy of Morell's extraction technic was checked first by comparing three per cent metaphosphoric acid alone with several other extractants and under several conditions of extraction and, second, by comparison with the hand-grinding technic of Burrell *et al.* (1940). The latter test was especially important because it was the technic most commonly used in the past and the one checked by animal assay (Harris and Olliver, 1942; Dunker *et al.*, 1942). The results agreed with those of Ponting (1943), Davis (1942), and Lucas (1944), which have since appeared, and it was concluded that Morell's extraction technic was satisfactory for cabbage.

While the present investigation was in progress, Loeffler and Ponting (1942) published another modification of the basic method containing further simplifications. Most important, they showed that it was possible to omit in practice the buffering of the extract and the extra dilution before the dye reaction. They established the use of oxalic acid as a cheap, stable substitute for metaphosphoric acid in the extraction. Comparison of their technic with Morell's showed close agreement on cabbage. The F value for the difference between the two methods was 5.77 ($F\ 0.05 = 5.99$).

A combination of Morell's (1941) and Loeffler and Ponting's (1942) technics with some modification was adopted as the standard technic in this investigation. Sampling was done by the composite 3-slice method and the extraction was made by blending two minutes in 200 ml of 0.5 per cent oxalic acid. The extract was filtered and the dye reaction carried out by adding suitable aliquots (0.5 to 2.0 ml) to 10 ml of dye solution (approximately 14.8 mg per liter) in colorimeter tubes. Readings were taken within ten seconds in an Evelyn colorimeter, though no evidence of drifting in the reading within 20 to 30 seconds was found with cabbage extracts. An excess of ascorbic acid was then added to the tube and a reading of residual color or turbidity was taken. Total dye readings were made with an equivalent volume of 0.5 per cent oxalic acid replacing the extract. All readings were taken with the colorimeter adjusted to an air blank (no tube in position) corresponding to total transmission (galvanometer reading = 100) for completely reduced dye and oxalic acid. Standardization was done substantially as described by Morell and a curve was fitted to the data by the method of least squares and the slope and intercept corrections were calculated. A dye concentration between those of Morell (1941) and Loeffler and Ponting (1942) was used to give a more suitable balance of working range and sensitivity for cabbage extracts. The range extended up to 75 mg per 100 g fresh tissue and covered the great majority of cabbage samples without an extra dilution. Calculation of the ascorbic acid content from the galvanometer

readings was made by the following formula, in which L_1 refers to the logarithm of transmission of dye plus solvent and L_2 to the logarithm of transmission of dye plus extract.

$$\frac{(L_1 - L_2) + 0.018}{0.0935} \times \frac{\text{vol. of dye} + \text{vol. of aliquot}}{1} \times \frac{\text{vol. of extract} + \text{vol. of tissue water}}{\text{vol. of aliquot}} \times \frac{100}{\text{wt. of sample}} \times \frac{1}{1000} = \text{mg/100 g}$$

The volume of tissue water was determined periodically from the dry weight and found to vary only slightly so that in practice 90 per cent of fresh weight could be used. Correction for turbidity, when necessary, was made as follows: the galvanometer reading for dye + solvent was multiplied by the turbidity reading divided by 100 and this value was used to look up L_1 in the L–G tables furnished with the instrument. In practice it was necessary to determine L_1 only for the few higher integral turbidity readings (99, 98, 97, 96, etc.) since turbidity of cabbage extracts was usually slight, and it was sufficiently accurate to make the correction corresponding to each L_2 only to the nearest whole number.

The standard technic was tested further to confirm the absence of significant amounts of dehydroascorbic acid and bound ascorbic acid. Analyses to include dehydroascorbic acid were made on aliquots of the standard extracts of fresh and stored cabbage including core, midrib, leaf, middle slice, and outer green leaf samples of the heads. Ten-ml aliquots of each sample were saturated with hydrogen sulphide by bubbling for 30 minutes, stored overnight at 6°C., and washed free of excess sulphides with a stream of oxygen-free nitrogen for four to six hours or until the extracts gave a negative test with lead acetate paper. Total apparent ascorbic acid was then determined as on the original aliquot. One group of 16 samples including several heads and regions of the head averaged 61.6 mg/100 g for ascorbic acid and 62.6 mg/100 g for total and the difference was non-significant ($F = 2.51$; $F_{0.05} = 4.54$). Another similar set averaged 65.9 and 67.8 mg/100 g respectively, and the difference was small but significant ($F = 6.34$). These differences, however, were probably within experimental error and certainly not large enough to warrant routine determination of dehydroascorbic acid in cabbage. Furthermore, there was no association of amount of dehydroascorbic acid with regions of head and no evidence was found for large amounts in green leaves of the head as reported by Günther (1943).

Two methods were used to test for appreciable quantities of bound ascorbic acid. Harris and Olliver's (1942) method was compared with the standard technic and found to average about five per cent higher, which was also probably within experimental error of comparison of alternate slices and likewise not a large enough difference to warrant routine determination of bound ascorbic acid. Another attempt was made to hydrolyze bound ascorbic acid by refluxing aliquots of extracts similar to De Eds' method (1943). Comparison of both reduced ascorbic acid and total ascorbic acid in the treated aliquot with the original showed less than two per cent dehydroascorbic acid and less than four per cent increase in total ascorbic acid on hydrolysis. It was concluded that no significant amounts of either dehydroascorbic or bound ascorbic acid were present in cabbage and no special precautions were taken to determine them.

It was important to have some estimate of the accuracy and the precision of the chemical part of the analysis to compare with sampling errors and tissue variation. The general evidence for the accuracy of the method was briefly reviewed above but it is difficult to make a really quantitative estimate. The best evidence, perhaps, is typified by Harris and Olliver's (1942) ratios of values for animal assay to chemical analysis for cabbage "water" and dried cabbage averaging 1.00 (0.93 to 1.07). Exclusive of the question of interfering substances and completeness of extraction, recovery data also gave some measure of accuracy, as well as precision, of the final steps of the analysis. Recovery measurements by Morell's (1941) technic were complicated, as he explains, by unavoidable variation in the ascorbic acid content within the head. Lower results than his were found, averaging 94 ± 3 per cent. Another method avoided this variation. A sample was blended for two minutes, a 15-ml aliquot was removed and replaced by 15 ml of an oxalic solution of suitable supplements of ascorbic acid, and the suspension blended two more minutes. Repeated tests without supplements showed no significant difference between two- and four-minute blending. Recovery by this method averaged 98 ± 2 per cent and seemed a better estimate of the recovery and precision than that of Morell (1941). Another estimate of precision alone for those steps following blending can be had from duplicate readings on extracts. The coefficient of variation for a large series of these on tissue ranging from 50 to 90 mg/100 g was 4.5 per cent.

It seemed likely, therefore, that the accuracy of the standard technic with cabbage was better than 95 per cent and may have approached 98 per cent of the ascorbic acid in the tissues. The precision appeared to be in the order of a coefficient of variation of five to seven per cent of the mean. This error of the chemical part of the analysis was distinctly smaller than other sources of error entering into the estimation of population means. Sampling errors for the 6-head samples most commonly used were measured by an average coefficient of variation of 10 to 15 per cent of the mean for the collection of standard varieties in 1943. Errors of within-head sampling were probably nearly as high. Choice of 6-head samples was a compromise between economy and sampling error allowable. A more refined chemical technic might have improved the precision and possibly

the accuracy of analysis but only at the expense of simplicity and greater analytical costs. The present modification was found a suitable compromise for large scale studies on cabbage.

Relation of variety to ascorbic acid content.—In 1942 and 1943 opportunities occurred to study the ascorbic acid variation in extensive varietal and breeding trials of cabbage at Madison and Kenosha, Wisconsin. Preliminary comparisons showed that sampling errors among replicate rows were consistently so much smaller than within rows that most of the sampling was done by random selection of six heads from single rows of 50 to 75 plants. Analyses were made either on the day of harvesting or after short periods of storage at 4°C., which had been shown to have no effect on ascorbic acid content.

TABLE 2. *Mean ascorbic acid content of 9 strains within 3 varieties of cabbage grown at Madison and at Kenosha, Wisconsin, in 1943.*

Variety	Strain	Ascorbic acid (mg/100 g) Madison	Kenosha
Wis. Hollander × Wis. Ballhead	8340M	48.0	44.8
	8340Y	52.0	52.8
	8341A	51.2	55.8
	8341P	48.1	48.9
Wis. Ballhead	3341B	61.1	63.6
Wis. All Seasons	2539M	59.0	61.8
	2542K	57.8	60.4
	2542M	60.0	65.8
	2542C	84.8	84.3

F values: for strains and varieties 41.7**; for locations, 2.40 (NS); for interaction, 0.44(NS). (** = significant at 1 per cent; NS = not significant).

In 1943, analyses were made on a series of the same nine strains of three varieties grown at Madison and at Kenosha to compare the relative effects of location and variety on ascorbic acid content (table 2). Analysis of variance showed a highly significant variation among strains and varieties and a non-significant difference between locations and for variety-location interaction. Apparently location and any associated environmental factors had little influence compared with genetic constitution in controlling ascorbic acid content in this experiment.

In 1943 an extensive list of standard varieties was grown at Madison, Wisconsin. Seed was sown out of doors early in May and plants were transplanted to the main field on June 14. In the results presented in table 3 the varieties are grouped according to their maturity season and the shape of head produced. It will be seen that the early, pointed group was highest in ascorbic acid as a whole. The varieties in the early, round group, all closely related genetically, were lower than those in the pointed group. The varieties in the mid-season, flat group were consistently lowest in ascorbic acid. In the late flat group, Late Flat Dutch and Winter Colma, both closely related to Early Flat Dutch, were lowest. Drumhead Savoy, a variety quite distinct from others in the group, was markedly higher in ascorbic acid. The late, round storage varieties also varied considerably. Neglecting environmental influences due to varying maturity ranges, the results showed, as has been indicated by others (Gould *et al.*, 1936; Burrell *et al.*, 1940; Murphy, 1942; Poole *et al.*, 1944), that varieties may differ significantly in their inherent capacities to produce ascorbic acid.

Effect of season upon ascorbic acid content.—Certain of the varieties listed in table 3 were also transplanted at earlier and/or later dates. The earliest transplanting, May 11, was from plants started in the cold frame. The main transplanting (June 14) was from plants grown out of doors. From a still later sowing out-of-doors plants were set on June 30. The ascorbic acid content of heads of the varieties as they came to maturity is given in table 4. It will be seen that the general tendency was for the August harvests to be higher than either the July or September harvests with little or no differences between September and October harvests. The differences were especially striking for July and August harvests of early types and were found significant in all but one case. However, in all harvests early pointed varieties were higher than early round ones. The drop in September compared with August harvests was significant in only two of the five cases for early and mid-season varieties. The differences between September and October harvests of late varieties were in no case significant. These results indicated rather strongly that the inherent capacity of a variety to build up ascorbic acid could be modified to some extent by the environment under which it was grown.

A study was made of a number of strains of Wisconsin Ballhead, Wisconsin Hollander, Bugner, and Wisconsin Ballhead × Wisconsin Hollander, all related late storage varieties, from the 1942 and 1943 crops, to compare the relative effects of changes from year to year and variety. Analysis of variance (table 5) showed a rather high interaction between strain and year and, consequently, a non-significant variation among strains compared with the interaction. Year variations, on the other hand, showed a highly significant increase in ascorbic acid content in 1943. The high interaction indicated that year to year effects had obscured any varietal differences in ascorbic acid content in these closely related strains.

The variations in ascorbic acid content among harvests of the same transplanting were scrutinized for evidence of the effect of several environmental factors including light energy, rainfall, temperature, and day length, to determine whether one or another had sufficiently striking influence, either over the whole growing season or during short periods before harvest, to exhibit some effect in the limited data available. The roughly similar ranges of values in early, mid- and late seasons argued against any simple relation between ascorbic acid

TABLE 3. *Relation of ascorbic acid to variety; lots transplanted on June 14.*

Seasonal and head type	Variety Name	Days from transplanting to harvest	Mean ascorbic acid mg/100 g[a]
Early; pointed	Jersey Wakefield	59	63.3
	Jersey Queen	71	71.0
	Charleston Wakefield	71	67.4
Early; round	Golden Acre	59	57.4
	Resident Detroit	56	54.7
	Copenhagen Market	56	54.7
	Racine Market	65	54.2
Mid-season; round	Marion Market	71	56.0
	Globe	71	55.1
	Glory of Enkhuizen	79	46.2
Mid-season; flat	Early Flat Dutch	79	47.3
	Allhead Early	81	47.5
	Allhead Early	89	46.8
	Allhead Select	81	43.1
	Allhead Select	89	42.3
Late; flat	Wisconsin All Seasons	95	51.1
	Late Flat Dutch	99	47.9
	Winter Colma	93	46.5
	Drumhead Savoy	107	61.4
Late; round	Wisconsin Ballhead	100	60.9
	Wisconsin Hollander	115	46.5
	Bugner	113	55.3
	Resistant Red Hollander	99	66.3

[a] LSD at 5 per cent level, 2.6; at 1 per cent level, 3.5.

content of varieties as a whole and temperature, day length, and long-period light energy (measured as mean daily total incident light energy for 20-day period preceding harvest), factors which tended to diminish in magnitude rather regularly through the growing season. Varieties obviously responded differently to different levels of these factors if, indeed, these were controlling ones.

TABLE 5. *Ascorbic acid content of 13 strains from 3 varieties of cabbage grown in 1942 and 1943.*

Variety	Strain	Mean ascorbic acid (mg/100 g) in[a] 1942	1943
Wis. Hollander × Wis. Ballhead	8340M	42.6	46.3
	8340T	43.6	49.3
	8340Y	48.8	51.9
	8341A	47.2	51.1
	8341M	55.4	49.6
	8341N	41.5	06.2
	8341P	40.4	48.1
Bugner	638A	44.6	51.7
	640M	38.6	47.4
	641A	38.4	51.1
	641M	40.3	51.4
	6411	48.2	55.4
Wis. Hollander	841A	51.1	55.1

[a] F values (using interaction term); for strains and vars., 1.08 (NS); for seasons, 15.4**.

One effect from transplanting data which may have been due to one of these factors was the tendency for several early varieties to give higher ascorbic acid values when harvested in August than when harvested in July. Lower rainfall in periods preceding the August harvests may have been responsible. A negative correlation between rainfall and ascorbic acid content was also reported for turnip greens by Reder *et al.* (1943).

No evidence was found under field conditions that the amount of light energy preceding harvests affected ascorbic acid content consistently as has been reported for several other crop plants (Murphy, 1938; Hamner and Maynard, 1942; Maynard and Beeson, 1943; Reid, 1943; Hansen and Waldo, 1944). Some varieties appeared to show negative correlations, some positive. The short-period effect of light energy was also tested by comparing early morning and late evening samples on clear days. Twelve alternate head samples were taken one hour after sunrise and again about two hours before sunset. Racine Market showed a small but significant increase from 55.0 to 61.0 ($F = 19.8$, $F_{0.05} = 4.30$) but Wisconsin Ballhead showed a non-significant increase of 62.5 to 66.3 ($F = 3.66$).

Relation of head maturity to ascorbic acid content.—The effect of maturity on the ascorbic acid content of fruit and vegetative crops has received considerable attention, for it might conceivably influence harvesting practices in the future. While the ascorbic acid content of young, physiologically ac-

TABLE 4. *Relation of time of harvesting to ascorbic acid content.*

Maturity type	Variety	Mean mg/100 g fresh tissue in plants harvested during the periods indicated[a] July 12–20	Aug. 9–24	Sept. 7–22	Sept. 29–Oct. 19
Early	Jersey Wakefield	55.1	63.3	...	...
	Jersey Queen	54.7	71.0	...	...
	Charleston Wakefield	...	67.4	57.7	...
	Golden Acre	46.8	57.4	...	...
	Resistant Detroit	49.6	54.7	52.1	...
	Copenhagen Market	42.7	54.7	...	...
	Racine Market	46.6	54.2	49.8	...
	Round Dutch	58.1	...	49.6	...
Midseason	Marion Market	53.8	56.0	52.7	...
	Globe	...	55.1	46.0	...
	Allhead Select	...	...	43.1	41.2
Late	Wisconsin All Seasons	...	...	51.1	53.8
	Wisconsin Ballhead	...	...	60.9	64.1
	Bugner	...	...	52.0	51.0

[a] LSD at 5 per cent level, 5.9; at 1 per cent level, 7.8.

tive tissue is generally higher than that of mature tissue (Schopfer, 1943), maturity in many crop plants has little practical effect. Wade and Kanapaux (1943) found no significant differences in snap beans from the small to large pod stage. Hamner and Maynard (1942) pointed out that, while partially grown green tomato fruits were lower than ripe ones, full-sized "mature green" fruit were about the same as the latter. In the case of cabbage, Murphy (1942) emphasized the progressive decline in ascorbic acid content of the head during formation, but it is not clear from her data that there were significant changes during final maturation of the head.

Several harvests were made of a number of standard varieties from the 1943 Madison plantings varying in average maturity from loose, immature to bursting, over-ripe heads. Comparison of these samples showed no consistent relation between ascorbic acid content and maturity, though significant differences often appeared between mature and immature samples. Similarly, analysis of variance between first and last harvests of 20 transplantings of 14 varieties showed no significant differences ($F = 2.69$, $F_{0.05} = 4.38$) for the varieties as a whole. In another line of approach 132 individual heads from 22 samples of 17 varieties classified as mature or immature were tested for association of ascorbic acid content and maturity by the chi-square method. The test showed no association (chi-square $= 2.60$, $P = 0.80$). On the whole differences between loose, immature and bursting, over-ripe heads were relatively small and may have been largely due to short-period environmental effects. Sufficient data for enough individual varieties were not available, however, to determine whether each might not have a characteristic behavior in this respect.

Relation of head size to ascorbic acid content.—Recent reports have shown a negative correlation of ascorbic acid content with fruit size in tomato (Hallsworth and Lewis, 1944) and apples (Batchelder and Overholser, 1936), and with head weight in cabbage (Poole *et al.*, 1944). In the last case, variance of ascorbic acid content due to strain, season, and replicate was compared with covariance on head weight. A similar analysis of some of the data from table 3 is given in table 6. It should be noted that "seasons" in this case were early and late transplantings and that material represented was of diverse genetic makeup. There were highly significant varietal, seasonal, and interaction effects based on the error term but only a slightly significant effect of season alone based on interaction. Reduction in sums of squares in the covariance analysis left highly significant varietal and interaction effects. This indicated that there was a real difference between varieties in ascorbic acid content over and above differences due to head weight. In contrast to Poole *et al.* (1944), the correlation for the error term which gave the measure of the average relationship between ascorbic acid content and head weight within varieties within a season was not significant.

Relation of soil fertility to ascorbic acid content.—Two trials of the effect of the fertility level on ascorbic acid content of Bugner variety were made at Kenosha, Wisconsin. In 1942 samples were analyzed from plots receiving no fertilizer and 2000 pounds per acre of 3-12-12 respectively, in each of three randomized blocks. The mean values of yield and ascorbic acid are given in table 7. Analysis of variance showed non-significant variation in ascorbic acid content for either levels or blocks though there was a large difference in yields. Similarly in 1943 analysis of duplicate blocks which received 2000 pounds and 250 pounds of 3-9-18 fertilizer

TABLE 6. *Relation of variety, head size and season to ascorbic acid content in cabbage.*

Variety	Mean head weight and ascorbic acid content: Early planting Head weight	Early planting Ascorbic acid	Late planting Head weight	Late planting Ascorbic acid	Early and late planting Head weight	Early and late planting Ascorbic acid
	grams	mg/100 g	grams	mg/100 g	grams	mg/100 g
Jersey Wakefield	1120	55.2	580	63.5	850	59.3
Jersey Queen	870	54.7	620	67.2	750	60.9
Golden Acre	1070	49.2	1200	57.0	1130	53.1
Resistant Detroit	1730	49.7	1510	52.2	1620	50.9
Racine Market	1870	46.7	1040	49.8	1450	48.3
Copenhagen Market	1660	43.0	1200	54.8	1430	48.9
Marion Market	1260	53.8	1660	56.2	1460	55.0
Round Dutch	930	58.0	980	49.7	950	53.8

Analysis of variance and covariance

	Variance: Degrees of freedom	Variance: Mean squares	Variance: F value Err.	Variance: F value Inter.	Covariance: Degrees of freedom	Covariance: Mean squares	Covariance: F value Corr. coeff.
Varieties	7	250.61	23.1[b]	1.85	6	98.1	8.41[b]
Seasons	1	605.1	55.7[b]	4.46[a]	0	.....	....
Vars. × Seasons	7	135.66	12.5[b]	...	6	108.41	9.30[b]
Error	80	10.86	...	...	79	11.66	—0.1693
Total	95	.....	...	...	94	.....	—0.6190[b]

[a] Significant at 5 per cent level.
[b] Significant at 1 per cent level.

showed no significant variation in ascorbic acid content with levels of fertility.

Inheritance of ascorbic acid content.—The evidence presented herein as well as that of others (Burrell *et al.*, 1940; Murphy, 1942; Poole *et al.*, 1944) indicate that hereditary factors are highly important in determining the differences between varieties in ascorbic acid content. Certain breeding lines derived from Wisconsin All Seasons were made the subject of special study. This variety has been in commercial use for many years. In 1943, when transplanted at Madison on June 14, the ascorbic acid value on September 17 was 51.1 mg/100 g fresh tissue; when transplanted on June 30 and harvested on September 29, the value was 53.8.

TABLE 7. *Yield and ascorbic acid content of cabbage grown on plots to which various amounts of fertilizer were added.*

Year and formula	Amount applied per acre	Yield	Mean ascorbic acid
	lbs.	tons per acre	mg/100 g[a]
1942	0	3.8	41.4
3-12-12	2000	14.5	42.6
1943	250	9.4	47.0
3-9-18	2000	13.7	50.7

[a] F values for ascorbic acid variance in 1942: blocks, 0.43; levels, 0.28; in 1943: blocks, 0.10; levels, 0.27.

When transplanted in the same year at Kenosha on June 22 and harvested on September 24, the ascorbic acid value was 56.7.

In the course of a selection and breeding program directed toward the fixing of certain other plant characters, a number of inbred lines from this variety had been acquired which were available for this work. Two lines derived from a single plant had been inbred for seven generations. These are designated herein as lines 15 and 38. A third line derived from a second plant had been inbred for five generations; this is designated herein as line C. Individuals from line 15 and C were hybridized and F_1 plants were allowed to sib-cross. A selfed progeny from an F_2 plant was designated as line 10. Selfed plant progenies from lines 10, 15 and 38, crosses between individual from these lines, and a sib-cross progeny from line C were grown in 1943 at Madison. The plants were transplanted on June 24; analyses were made in September. The results are given in table 8.

Selfed progenies from line 15 averaged within the range of the parent variety. Selfed progenies from line 38 averaged slightly lower. The average of F_1 hybrid lines from crosses between individuals from line 15 and line 38 was between the average for the selfs from the two parents. However, there was enough variation within each group to invalidate the significance of any variation from that of the parent variety. Line C was distinctly higher than the parent variety and than any progeny from

the other inbred lines. Line 10 was at the upper range of the parent variety. It will be recalled that plants 263 and 268 in line 10 were F_2 individuals from a cross between lines C and 15. When sister plants of 263 and 268 were crossed with individuals from line 38, the hybrid progenies tended to be in the upper range of the parent variety. The results of the 1943 trials served to show that in four distinct inbred lines from the parent variety, progenies from three lines were relatively close to the parent variety and variation between individual progenies may have been due to environmental effects. In the fourth line (line C), however, the indications were strong that through selection an inherent higher plane of ascorbic acid content had been established.

Individuals from certain of the progenies were selected in the autumn of 1943 and, during the ensuing winter, seed was propagated in the greenhouse by sib-crosses of individuals within progenies and by crossing individuals from high and low ascorbic acid lines. The progenies thus secured were grown in the field in 1944 at Madison and ascorbic acid determined at maturity. The results, together with the 1943 ascorbic acid determination on the progenies from which parents were selected, are given in table 9. It may be seen that in every case the progenies from sib-crosses between individuals selected from low ascorbic acid progenies of 1943 were approximately equal to the parent progenies in ascorbic acid content. Likewise the progenies derived from individuals selected from the high ascorbic acid line were at the same level of ascorbic acid as the parent progeny. This was evidence that inbreeding had led to a fair degree of fixity of ascorbic acid content in these lines. In each of five hybrids between high and low lines the ascorbic acid content of the F_1 progeny was approximately midway between that of the respective parents.

Discussion.—The time is clearly past when limited, exploratory analyses of ascorbic acid in miscellaneous foodstuffs suffice as nutritional guides. The wide variations reported and the many factors apparently involved require more systematic study. First, sufficient attention has not always been given to adequate error control in sampling. Variation in the ascorbic acid content in the tissues of cabbage heads illustrates a possible source of discrepancy in the results of different laboratories. There is a tendency for the ascorbic acid content to be lowest in the white inner leaves and to be progressively greater in partially green outer leaves of the head and the outer, older leaves below the head. The content of the last mentioned may be three to four times as high as that of the white inner leaves. The differences in commercial harvesting practices in various areas may thus alter the proportion of green tissue included and the average for the head. Similarly, the method of sampling the head including more or less of the core or the outer green tissue can affect the average.

Table 8. *The ascorbic acid content of progenies derived from four inbred lines within Wisconsin All Seasons.*

Line number	Plant number	Generation	Mean ascorbic acid (mg/100 g tissue)
	Wisconsin All Seasons (commercial)		51.1–56.7
10	263	F_1 self	57.0
	268	F_1 self	61.3
	Average	F_1 self	59.2
15	278	F_1 self	55.1
	280	F_1 self	48.1
	281	F_1 self	53.9
	Average	F_1 self	52.4
38	291	F_1 self	50.5
	292	F_1 self	42.8
	293	F_1 self	42.6
	294	F_1 self	50.7
	295	F_1 self	48.3
	298	F_1 self	49.9
	Average	F_1 self	47.5
15:38	278 × 295	F_1 hybrid	51.5
	272 × 296	F_1 hybrid	49.1
	293 × 272	F_1 hybrid	48.5
	274 × 297	F_1 hybrid	47.2
	Average	F_1 hybrid	49.1
10:38	261 × 297	F_1 hybrid	54.1
	263 × 293	F_1 hybrid	46.1
	267 × 298	F_1 hybrid	51.5
	290 × 266	F_1 hybrid	51.9
	291 × 261	F_1 hybrid	56.3
	295 × 265	F_1 hybrid	62.2
	296 × 260	F_1 hybrid	56.3
	297 × 264	F_1 hybrid	60.4
	Average	F_1 hybrid	54.9
Average of lots from lines 10, 15 and 38			52.0
C	244–255	F_1 sib	82.9

Apart from analytical methods, it is difficult to rank accurately even the common varieties for which analyses have appeared from several laboratories. This variation is not always evidence of environmental influence but often may reflect variation in the genetic constitution of seed stocks from different sources. Occasional comparison of ascorbic acid contents from various sources of seed within one variety in the 1943 tests bore out this conclusion. Future work should take into account the possibility of genetic variation in strains within an individual variety.

There are not sufficient data as yet to appraise adequately the role of environment in the ascorbic acid content of cabbage in the field. The data in this investigation serve to emphasize the importance of seasonal differences, both in years and times of the year, and it is suggested that rainfall may occasionally be a controlling factor. Failure to find definite evidence for the influence of light energy level probably points only to the greater complexity of its effect on a head crop like cabbage in comparison with the more directly exposed fruit and leaf crops. Whether genetic or environmental differences are the more important in controlling ascorbic acid content of cabbage is not easy to answer. The data

TABLE 9. *Ascorbic acid content of progenies derived from high and low ascorbic acid lines of Wisconsin All Seasons and in F_1 hybrids from crosses between lines.*

Progenies from which parents were selected		Parent and hybrid progenies		
Lot number	Mean ascorbic acid (1943)	Plant number	Generation	Mean ascorbic acid (1944)
	mg/100 g			mg/100 g
292 self	42.8	328	F_1 sib	44.1
2542C	82.9	484	F_1 sib	80.5
		328 × 484	F_1 hybrid	68.0
272 × 296	49.1	310	F_1 sib	52.5
2542C	82.9	494	F_1 sib	91.7
		310 × 494	F_1 hybrid	65.1
274 × 297	47.2	312	F_1 sib	47.5
2542C	82.9	497	F_1 sib	82.7
		312 × 497	F_1 hybrid	66.6
290 × 266	51.9	315	F_1 sib	49.8
2542C	82.9	496	F_1 sib	83.5
		315 × 496	F_1 hybrid	73.9
291 × 261	56.3	323	F_1 sib	54.4
2542C	82.9	486	F_1 sib	89.9
		323 × 486	F_1 hybrid	78.1
Average of low ascorbic acid parents				49.7
Average of high ascorbic acid parents				85.7
Average of F_1 hybrids				70.3

acquired in the present investigation seem to support the conclusion of Poole *et al.* (1944) that the former was more important. However, tests over a greater range of environments might show even wider variation within varieties. More adequate information on these problems can come only with collaborative work of several stations using uniform methods and seed stocks.

The survey of the relative level of ascorbic acid content in the inbred lines of Wisconsin All Seasons showed that it was possible by selection to secure lines in which the ascorbic acid content was much higher than the parent stock. The high ascorbic acid character was apparently well fixed and resulted in intermediate levels in the F_1 from crosses with low acid lines. This indicates that by hybridization the high acid character may be introduced into other desirable types. That fairly high ascorbic acid content can be combined with high yield, satisfactory type, and increased disease resistance has already been indicated (Walker, 1944). We may expect, therefore, to see the development and adoption of cabbage varieties greatly improved in nutritive value, disease resistance, and productivity.

SUMMARY

The method of sampling cabbage heads for ascorbic acid analysis was found to be very important. A standard method of sampling was adopted, which is described. A combination of Morell's and of Loeffler and Ponting's technic of extraction and analysis with some modification, was worked out for the problem in hand.

No significant amounts of either dehydroascorbic acid or bound ascorbic acid were found in cabbage heads.

Significant differences between varieties of cabbage were found to occur independently of fluctuation due to one or another environmental factor. Varieties in the early pointed group were in general higher than those in the early round and mid-season round groups. The mid-season flat varieties and some of the late flat varieties were the lowest in acid content.

In a series of transplantings and harvests there was a tendency for heads in the August harvest to be higher than in either July or September harvest.

In a series of closely related strains there was a highly significant increase in ascorbic acid content in one season over that in the previous season.

No simple relation was found between ascorbic acid content and temperature, day length, or long-period light energy. The higher ascorbic values in the August harvest, mentioned above, may have been due to low rainfall preceding harvest.

No correlation was found between stage of head maturity and ascorbic acid content.

In contrast to previous reports the correlation for the error term which gave the measure of the average relationship between ascorbic acid content and head weight within varieties within a season was not significant.

No correlation between soil fertility, yield and ascorbic acid was noted.

A study was made of inbred lines of Wisconsin All Seasons, a variety which contained 51 to 56 mg/100 g ascorbic acid. Lines were derived in

which the acid content was not far removed from that of the parent variety, while one line was derived which yielded 80 to 91 mg/100 g. When individuals from the high yielding line were crossed with individuals from low yielding lines, the yields in the F_1 progenies were consistently intermediate between the two parents. The possibility of increasing the ascorbic acid content of standard varieties and combining it with disease resistance is discussed.

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VOLUME–SHAPE RELATIONSHIPS IN VARIANT FOAMS. A FURTHER STUDY OF THE RÔLE OF SURFACE FORCES IN THREE–DIMENSIONAL CELL SHAPE DETERMINATION [1]

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IT HAS been suggested for a long time by various investigators that surface forces play a part in three-dimensional cell shape determination and, consequently, in plant and animal development. Recently the importance of such forces has been analyzed through a study of bubbles of uniform size in a specially constructed foam. While investigation of such an almost perfect foam serves to throw light on the general and theoretical aspects of this whole problem, no tissue has been studied in which the cells, like the bubbles, were all of equal volume. In order to achieve more direct comparisons between non-living and cellular systems, an additional series of experiments has been conducted in which the volumes of the bubbles in a foam were varied in specific and controlled ways. The data assembled should make possible, first, a further comparison between cells in tissues and bubbles in foam; secondly, and this is of greater importance, such data should permit a more complete analysis of the part which surface forces play in the establishment of the three-dimensional shapes of cells, and therefore, inevitably, in certain aspects of the development of plants and animals.

LITERATURE REVIEW.—An extensive survey of the literature dealing with soap films and their bearing on the general subject of cell shapes has recently been made (Matzke, 1946). Since the present article is in a sense a continuation of that one, in which complete citations are given, only those aspects of the literature which deal specifically with the problem outlined in the preceding paragraph will be mentioned here. All the cells which have been studied and modeled have varied in size. Data are available for comparison with soap bubbles especially in the following tissues which have been investigated by various authors: 100 elder (*Sambucus canadensis*) pith cells (Lewis, 1923), 100 human fat cells (Lewis, 1925), 50 tadpole precartilage cells (Lewis, 1933), 100 *Eupatorium purpureum* pith cells (Marvin, 1939b), 100 *Angiopteris evecta* petiole parenchyma cells (Higinbotham, 1942), 100 *Citrus grandis* carpel vesicle cells (Dodd, 1944), 100 *Eupatorium perfoliatum* pith cells (Marvin, 1944), 150 *Ailanthus glandulosa* pith cells (Hulbary, 1944), 600 *Anacharis densa* (Elodea) cortical cells (Hulbary, 1944). The data on soap films gain added significance when they are compared and contrasted with the cellular statistics presented by these investigators, as shown below. A system in which the effect of surface forces in shape determination is not of major significance had been analyzed previously; lead shot of uniform size were compressed in a steel cylinder to eliminate interstices, and the pellets were then picked apart and the number of contacts recorded by Marvin (1939a). Similar investigations on large and small shot, mixed in varying proportions and compressed to eliminate interstices, were made by Matzke (1939). The work on large and small soap bubbles mixed in varying proportions described below is a counterpart of this earlier study on the large and small shot. In the soap films, in contrast to the shot, surface forces are obviously of marked importance. Comparisons of two such systems, composed of units of varying sizes, with cells in tissues, the cells also not uniform in size, should throw further light on the part which surface forces play in three-dimensional cell shape determination. The general topic of cell shapes has been reviewed in recent years by Thompson (1942), Lewis (in Bremer-Weatherford, 1944), and Matzke (1940).

MATERIAL AND METHODS.—The technique used in the three sets of experiments described below was essentially similar to that described for bubbles of uniform size (Matzke, 1946), except that in the present work bubbles of two different sizes were made, one by one, and mixed in definite and known proportions. The soap solution, as previously, consisted of the following parts: triethanolamine oleate 7.5 g., glycerine 34 g., and distilled water 58.5 g. These materials were shaken together and then allowed to stand for several weeks.

Each bubble was made separately by setting the plunger of a Yale tuberculin syringe, graduated to 1/100 cc., so that the syringe contained .05 cc. or .4 cc. of air (depending upon whether a small or a large bubble was to be made), then touching the tip of the syringe in the soap solution, and pushing down the plunger. In that way it was possible to make bubbles of fairly precise and known volumes. After each bubble was "blown," the tip of the syringe was wiped on cheesecloth to remove excess soap solution. The bubbles were placed, one by one, into a cylindrical dish having an inside diameter of 6 cm. and an inside height of 6.5 cm., with a volume of 188 cc. The inside of the dish was moistened beforehand with the soap solution. A glass plate was used as a cover. The dish was never completely filled, so that the bubbles did not come in contact with the glass plate on top even when temperature increases resulted in expansion. The dish was rotated from time to time, to keep the solution distributed as uniformly as possible and to allow the bubbles to slip and adjust themselves; if the bubbles were to be kept for some time, the dish was rotated on its long axis on a clinostat. Although the bubbles would last for days, they were always studied as soon as possible, after a brief rotation, on the same day on which they were made. The general appearance of the dish with bubbles was similar to that shown in figures 1 and 2 of a

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previous publication (Matzke, 1946), except that in the present work the bubbles varied in volume, while there they were all uniform.

The bubbles were examined, as before, with a Spencer binocular dissecting microscope with 1X paired objectives and 6X paired oculars. With this type of microscope it was possible to single out any bubble in a mass, by focusing; those above and below had little obscuring effect if the films were thin enough. In this way the number and kinds of faces of each bubble could be recorded, as indicated in the tables below.

Only "central" bubbles were studied in this work. By central are meant those which were removed by at least three other bubbles (large or small) from the top, bottom, and sides of the mass. In other words, if the dish was filled (or nearly so) with bubbles, only those in a central core were used for compilation; no bubble was tabulated unless it was at least the fourth one from any peripheral surface.

During the course of the three sets of experiments described below, the dish was filled 19 times, with a total of 32,181 bubbles, each of which was made individually with a measured volume and placed into the dish separately. Since data were assembled on only 300 bubbles in all, 150 small ones and 150 large ones, in the three sets of experiments, it may seem strange that so many had to be made. However, the number of large bubbles separated sufficiently from the top and sides was limited; the bubbles were studied only directly after the dish was filled; if any breaking occurred while the data were being tabulated, the whole mass was discarded, and the dish was filled over again. These limitations made it necessary to refill the dish a large number of times. The bubbles would last for a long time, often for days, and the large ones especially could be identified day after day. Slight changes would not infrequently occur from day to day, even on the clinostat, so that the system was never completely static, although it soon became relatively stable. Also, the large bubbles would tend to increase in volume, and the small ones would tend to decrease. For these reasons the data were always assembled on the day on which the bubbles were made.

The illustrations shown in figures 1 to 60 are drawings of individual bubbles made with a Leitz camera lucida attached to a Spencer binocular dissecting microscope with 1X objective and 6X ocular. The drawings were done by plotting the corners of each face and connecting them with straight lines. In this way the fine curvatures of the films and edges were not reproduced; but to show these curvatures would have been quite difficult, and this method was adopted, reluctantly, merely for expediency. Since the bubbles drawn were not necessarily at the same level in the dish, and since the drawing board was kept at constant height, the magnifications were approximately but not all precisely the same.

DATA.—The work here presented was performed in three sets of experiments, which for convenience will be termed experiment 1, experiment 2, and experiment 3. In experiment 1, two small bubbles, each with a volume of .05 cc., were put into the dish for every large one. Each of the large bubbles had a volume of .4 cc. The theoretical diameter of these bubbles would be .457 cm. for the small ones, and .914 cm. for the large ones; both were subject to pressure and contact. Each bubble was placed into the dish separately, with no effort at spacing or arrangement. The small and large ones were put in approximately in sequence. From time to time the dish was rotated to distribute the soap solution, which tended to collect in the bottom, uniformly throughout the dish. This allowed slipping and readjustments of the bubbles. The results of experiment 1 are shown in tables 1 and 4 and in figures 1 to 20.

In order to obtain the data presented for experiment 1, the dish was filled—not to its maximum capacity—six times, in the course of which a total of 3,860 small and 1,930 large bubbles were "blown."

The technique used in experiment 2 was similar to that in experiment 1, except that 8 small bubbles were made for each large one. The results are presented in tables 2 and 5, and in figures 21 to 39. In this case the dish was filled seven times with a total of 10,024 small and 1,253 large bubbles.

In experiment 3, 32 small bubbles were placed into the dish for each large one, with the results as indicated in tables 3 and 6 and in figures 40 to 60. In this experiment the dish was filled six times, with a total of 14,656 small and 458 large bubbles.

In experiment 1, as shown in table 1, the number of contacts on the small bubbles varied from 7 to 12, with an average of 9.68. Of the total of 50 small bubbles studied, 35 had 9 or 10 contacts. Actually, therefore, the range in variation was not very great. The average number of contacts of the large bubbles was 20.42, with a range from 17 to 24. Of the 50 large bubbles studied, 26 had 20 or 21 contacts, so that there was a considerable degree of constancy here, too.

On the basis of the 50 small and 50 large bubbles tabulated in experiment 1, with twice as many small as large ones, the average number of contacts of all the central bubbles can be computed to be 13.26. This compares with an average of 13.0 for compressed lead shot under parallel conditions (Matzke, 1939) and with 13.70 for bubbles all of uniform volume (Matzke, 1946), and with 14.17 for lead shot of uniform volume (Marvin, 1939a).

From the data presented above, it is evident that when small and large bubbles were mixed in the proportions indicated, the number of faces on the small bubbles dropped from an average of 13.70 to 9.68, while the number on the large bubbles rose from an average of 13.70 to 20.42. The general averages were similar to those of lead shot compressed to eliminate interstices in parallel experiments, but there were differences which will be pointed out below. Figures

TABLE 1. *Number of faces on bubbles. Experiment 1. Ratio of volume of each small to each large bubble 1:8 (.05 cc. to .4 cc.). Ratio of number of small to large bubbles 2:1.*

Data on 50 small bubbles			Data on 50 large bubbles		
No. of faces per bubble	No. of bubbles	Total faces	No. of faces per bubble	No. of bubbles	Total faces
7	1	7	17	1	17
8	4	32	18	5	90
9	17	153	19	6	114
10	18	180	20	14	280
11	8	88	21	12	252
12	2	24	22	10	220
	—	—	23	0	0
	50	484	24	2	48
				—	—
				50	1021
Average number of faces per bubble $\frac{484}{50} = 9.68$			Average number of faces per bubble $\frac{1021}{50} = 20.42$		

1 to 10 illustrate small bubbles, while figures 11 to 20 show large bubbles in experiment 1.

In experiment 2 the conditions were similar, but the number of small to large bubbles was 8:1. As shown in table 2, the average number of faces on the small bubbles here was 11.90, with a range from 9 to 14 contacts. Of the 50 small bubbles studied, 18 had 12 faces, and 42 had 11 to 13 contacts. Here again, then, the range was narrow. The 50 large bubbles in this experiment averaged 27.34 contacts, with a range from 24 to 31; and 24 of the 50 had 26 to 28 faces. Thus, they also were fairly uniform.

On the basis of the data in table 2, the average number of faces of all the bubbles in the dish at one time in experiment 2 can be computed, and is found to be 13.62. This is close to the average of 13.55 for compressed lead shot under similar conditions.

If the data in experiment 2 are compared with those of experiment 1, it is seen that the average number of contacts of the small bubbles was closer to 13.70 (the average for bubbles of uniform size), while the average for the large bubbles was farther away from 13.70. In this experiment large bubbles were obviously less frequently in contact with each other, and more commonly in contact with small bubbles than in experiment 1. Figures 21 to 30 illustrate small bubbles, and figures 31 to 39 show large bubbles in this experiment.

In experiment 3 there were 32 small bubbles for every large one. From table 3 it can be seen that the average number of contacts on the small bubbles was 13.20, with a range from 11 to 17, and 41 of the 50 bubbles had 12 to 14 faces. The average for the large bubbles was 30.06, with a range from 27 to 33, and 35 of the bubbles had 29 to 31 faces.

By computation, as before, the average number of contacts of all the bubbles in the dish at one time in experiment 3 is found to be 13.71. This is obviously

TABLE 2. *Number of faces on bubbles. Experiment 2. Ratio of volume of each small to each large bubble 1:8 (.05 cc. to .4 cc.). Ratio of number of small to large bubbles 8:1.*

Data on 50 small bubbles			Data on 50 large bubbles		
No. of faces per bubble	No. of bubbles	Total faces	No. of faces per bubble	No. of bubbles	Total faces
9	1	9	24	4	96
10	3	30	25	7	175
11	14	154	26	6	156
12	18	216	27	10	270
13	10	130	28	8	224
14	4	56	29	5	145
	—	—	30	9	270
	50	595	31	1	31
				—	—
				50	1367
Average number of faces per bubble $\frac{595}{50} = 11.90$			Average number of faces per bubble $\frac{1367}{50} = 27.34$		

TABLE 3. *Number of faces on bubbles. Experiment 3. Ratio of volume of each small to each large bubble 1:8 (.05 cc. to .4 cc.). Ratio of number of small to large bubbles 32:1.*

Data on 50 small bubbles			Data on 50 large bubbles		
No. of faces per bubble	No. of bubbles	Total faces	No. of faces per bubble	No. of bubbles	Total faces
11	1	11	27	2	54
12	16	192	28	5	140
13	16	208	29	10	290
14	9	126	30	13	390
15	6	90	31	12	372
16	1	16	32	7	224
17	1	17	33	1	33
	50	660		50	1503

Average number of faces per bubble $\frac{660}{50} = 13.20$

Average number of faces per bubble $\frac{1503}{50} = 30.06$

close to the average of 13.8 for the parallel lead shot experiment, and it is almost identical with the average number of contacts for central bubbles of uniform volume, 13.70. Drawings of small bubbles in experiment 3 are shown in figures 40 to 49, while figures 50 to 60 illustrate large bubbles.

In experiment 3 the average for the small bubbles was closer to 13.70 than in either of the other two experiments, while the average for the large bubbles was farther from that number than in either of the other experiments. Here the small bubbles were more frequently in contact with small bubbles, while the large ones were more often completely or nearly completely surrounded by small bubbles.

TABLE 4. *Data on kinds of faces of bubbles. Experiment 1. Ratio of volume of each small to each large bubble 1:8 (.05 cc. to .4 cc.). Ratio of number of small to large bubbles 2:1.*

Data on 50 small bubbles		Data on 50 large bubbles	
No. of sides per face	No. of faces	No. of sides per face	No. of faces
4	159	4	115
5	282	5	498
6	43	6	288
	484	7	113
		8	6
		9	1
			1021

The results of these experiments are shown graphically in figure 61, in which AA is the curve for bubbles of uniform diameter, previously published; BB represents the data of table 1 (experiment 1) on small bubbles, B′B′ the data of table 1 (experiment 1) on large bubbles; CC and C′C′ are curves for the data on small and large bubbles, respectively, in table 2 (experiment 2), and DD and D′D′ for the data on small and large bubbles, respectively, in table 3 (experiment 3). It is evident that curves BB, CC, and DD get progressively closer to AA, while curves B′B′, C′C′, and D′D′ get progressively farther away from AA.

TABLE 5. *Data on kinds of faces of bubbles. Experiment 2. Ratio of volume of each small to each large bubble 1:8 (.05 cc. to .4 cc.). Ratio of number of small to large bubbles 8:1.*

Data on 50 small bubbles		Data on 50 large bubbles	
No. of sides per face	No. of faces	No. of sides per face	No. of faces
4	118	4	57
5	370	5	625
6	101	6	557
7	6	7	118
		8	9
	595	9	1
			1367

TABLE 6. *Data on kinds of faces of bubbles. Experiment 3. Ratio of volume of each small to each large bubble 1:8 (.05 cc. to .4 cc.). Ratio of number of small to large bubbles 32:1.*

Data on 50 small bubbles		Data on 50 large bubbles	
No. of sides per face	No. of faces	No. of sides per face	No. of faces
4	96	4	36
5	412	5	624
6	148	6	749
7	4	7	92
		8	2
	660		1503

The data presented above deal with the number of faces on each bubble. In tables 4, 5, and 6, the kinds of faces—that is, quadrilateral, pentagonal,

TABLE 7. *Combinations of faces found in 150 small bubbles, from experiment 1, experiment 2, experiment 3, and similar (matching) combinations in bubbles, shot, and cells.*

Number of sides per face					Number of bubbles						Similar (matching) combinations in						
4	5	6	7	Total faces	Exp. 1	Exp. 2	Exp. 3	Total bubbles found	Illus. in fig.	400 periph. bubbles	600 central bubbles	150 small shot	104 periph. shot	624 central shot	550 undif. cells	600 Elodea cells
5	2	..	..	7	1	..	..	1	1	2	..	..	..	..	1	73
4	4	..	..	8	4	..	..	4	2	2	..	4	2	..	2	6
3	6	..	..	9	8	1	..	9	3, 21	2	..	11	2	..	3	13
4	4	1	..	9	8	..	..	8	4	11	..	1	4	..	2	15
5	2	2	..	9	1	..	..	1	5	4	..	2	1	..	8	3
2	8	..	..	10	3	..	..	3	6	3	..	3	..	..	6	..
3	6	1	..	10	14	2	..	16	7, 22	29	..	7	12	1	7	..
4	4	2	..	10	1	..	..	1	8	47	..	7	12	..	17	54
5	3	1	1	10	..	1	..	1	..	6	..	..	1	..	3	1
2	8	1	..	11	3	8	1	12	9, 23	35	..	2	2	4	5	..
3	6	2	..	11	4	4	..	8	10, 24	67	2	3	8	..	14	1
4	4	3	..	11	1	1	..	2	25	22	..	2	7	3	16	..
4	5	1	1	11	..	1	..	1	..	22	..	..	1	..	2	11
..	12	..	..	12	1	4	4	9	40	11	50	1	..	3	..	..
2	8	2	..	12	..	4	6	10	26, 41	19	15	2	..	10	9	..
3	6	3	..	12	1	4	4	9	27	22	7	5	2	9	14	..
4	4	4	..	12	..	3	1	4	..	9	1	2	1	3	16	5
4	5	2	1	12	..	3	1	4	..	3	..	..	1	..	7	..
1	10	2	..	13	..	5	7	12	28	17	118	2	..	5	1	..
2	8	3	..	13	..	1	3	4	42	5	19	3	..	12	6	..
3	6	4	..	13	..	2	4	6	29, 43	3	36	7	..	16	20	..
4	4	5	..	13	..	1	1	2	..	..	4	3	..	7	10	2
2	9	1	1	13	..	..	1	1	..	2	..	..	..	..	2	..
3	7	2	1	13	..	1	..	1	..	3	1	1	..	2	4	..
..	12	2	..	14	..	2	..	2	30	1	39	..	..	2	..	..
1	10	3	..	14	..	..	1	1	44	2	73	..	1	3	1	..
2	8	4	..	14	..	2	4	6	45	3	64	3	..	17	5	..
3	6	5	..	14	..	..	3	3	46	..	17	1	..	16	5	..
4	4	6	..	14	..	..	1	1	47	..	12	1	..	13	9	..
..	12	3	..	15	..	..	3	3	48	..	21	..	..	1	..	..
1	10	4	..	15	..	..	1	1	49	..	35	1	..	3	1	..
3	7	4	1	15	..	..	1	1	..	..	5	1	..	7	6	..
4	5	5	1	15	..	..	1	1	..	..	2	1	..	4	9	..
1	10	5	..	16	..	..	1	1	..	..	4	..	..	..	..	..
..	12	5	..	17	..	..	1	1	..	..	..	..	..	..	..	..
					50	50	50	150		352	525	76	57	141	211	184

TABLE 8. *Most commonly occurring combinations of faces in small bubbles, and similar (matching) combinations in bubbles, shot, and cells.*

Number of sides per face						Similar (matching) combinations in						
4	5	6	Total faces	Total bubbles found	Illus. in fig.	400 periph. bubbles	600 central bubbles	150 small shot	104 periph. shot	624 central shot	550 undif. cells	600 Elodea cells
3	6	1	10	16	7, 22	29	..	7	12	1	7	..
2	8	1	11	12	9, 23	35	..	2	2	4	5	..
1	10	2	13	12	28	17	118	2	..	5	1	..
2	8	2	12	10	26, 41	19	15	2	..	10	9	..
3	6	.	9	9	3, 21	2	..	11	2	..	3	13
.	12	.	12	9	40	11	50	1	..	3	..	..
3	6	3	12	9	27	22	7	5	2	9	14	..
1	4	1	9	8	4	11	..	1	4	..	2	15
3	6	2	11	8	10, 24	67	2	3	8	..	14	1
3	6	4	13	6	29, 43	3	36	7	..	16	20	..
2	8	4	14	6	45	3	64	3	..	17	5	..
				105		219	292	44	30	65	80	29

hexagonal, etc.—in experiments 1, 2, and 3, respectively, are tabulated. In experiment 1, for instance, the 50 small bubbles tabulated had a total of 159 quadrilateral faces, 282 pentagonal faces, and 43 hexagonal faces; the kinds of faces in the large bubbles are similarly given. It is obvious from these three tables that in the small bubbles pentagonal faces were more common than all others combined; there were 1,739 faces on the 150 small bubbles in the three experiments, of which 1,064 were 5-sided. In the large bubbles there were 3,891 faces in all, of which 1,747 were pentagonal. The pentagonal faces were more numerous than any other kind, but in the large bubbles they were not more abundant than all others combined. The faces were not necessarily plane, nor were the sides always straight lines.

These results are shown graphically in figure 62. The predominance of pentagonal faces in the small bubbles is clearly evident there; in the large bubbles pentagonal faces were most numerous in experiments 1 and 2, while hexagonal faces occurred most frequently in experiment 3. The range of types of faces was definitely narrow in all cases.

The 150 small bubbles in the three experiments occurred in 35 different combinations of faces (table 7); the 150 large bubbles in these same experiments were found in 105 combinations of faces. Since there was such a large number of these latter, with relatively little matching data from previous investigations, they are not being published in their entirety. However, 30 of these large bubbles are illustrated in figures 11 to 20, 31 to 39, and 50 to 60, and their combinations are recorded in tables 9, 10, and 11.

An inspection of table 7 reveals that the combination that was found most often, of the 35 which occurred in the 150 small bubbles, was 3-6-1—that is, 3 quadrilateral faces, 6 pentagons, 1 hexagon. This 10-faceted foam cell was recorded 16 times and is illustrated in figures 7 and 22. Two of the combinations occurred 12 times each—2-8-1 (fig. 9, 23), and 1-10-2 (fig. 28). There were 10 bubbles with the combination 2-8-2 (fig. 26, 41), and 9 with the combinations 3-6-3 (fig. 27), and 3-6-0 (fig. 3, 21). The pentagonal dodecahedron 0-12-0 (fig. 40) was also found nine times. These 7 combinations, of the total 35, accounted for 77 bubbles, or more than half; but at the same time none predominated overwhelmingly, so that no one of these was an outstanding "type." The other most common combinations (table 8) were 4-4-1 (fig. 4) and 3-6-2 (fig. 10, 24), both of which occurred eight times; and 3-6-4 (fig. 29, 43) and 2-8-4 (fig. 45), both of which occurred six times. These 11 most common combinations accounted for 105 of the 150 small bubbles recorded, or 70 per cent.

From table 7 it is obvious that certain combinations among the commoner ones recurred in all three experiments, and that a considerable number were recorded in two of the three experiments. In table 8 it can be seen that the commonest combinations never had more than three kinds of faces—quadrilateral, pentagonal, and hexagonal—and that one, the pentagonal dodecahedron, had just one kind of face—all pentagons. An inspection of table 7 reveals further that, in general, the small foam cells with the largest numbers of pentagonal faces, and hence with the smallest numbers of quadrilateral and hexagonal faces, occurred most frequently. Those combinations with large numbers of quadrilateral faces, with large numbers of hexagonal faces, and with heptagonal faces were found infrequently.

Figure 1, with 5 quadrilateral and 2 pentagonal faces, is really a modified pentagonal prism. The foam cell illustrated in figure 2, with 4 quadrilateral and 4 pentagonal faces, achieves a certain balance, as do the following combinations: 0-12-0 (fig. 40), 2-8-2 (fig. 26, 41), 3-6-3 (fig. 27), and 4-4-4 (not illustrated).

Of the 150 large bubbles tabulated, one combination—0-13-16-1—occurred seven times (table 10). This 30-faced foam cell, which is illustrated in figure

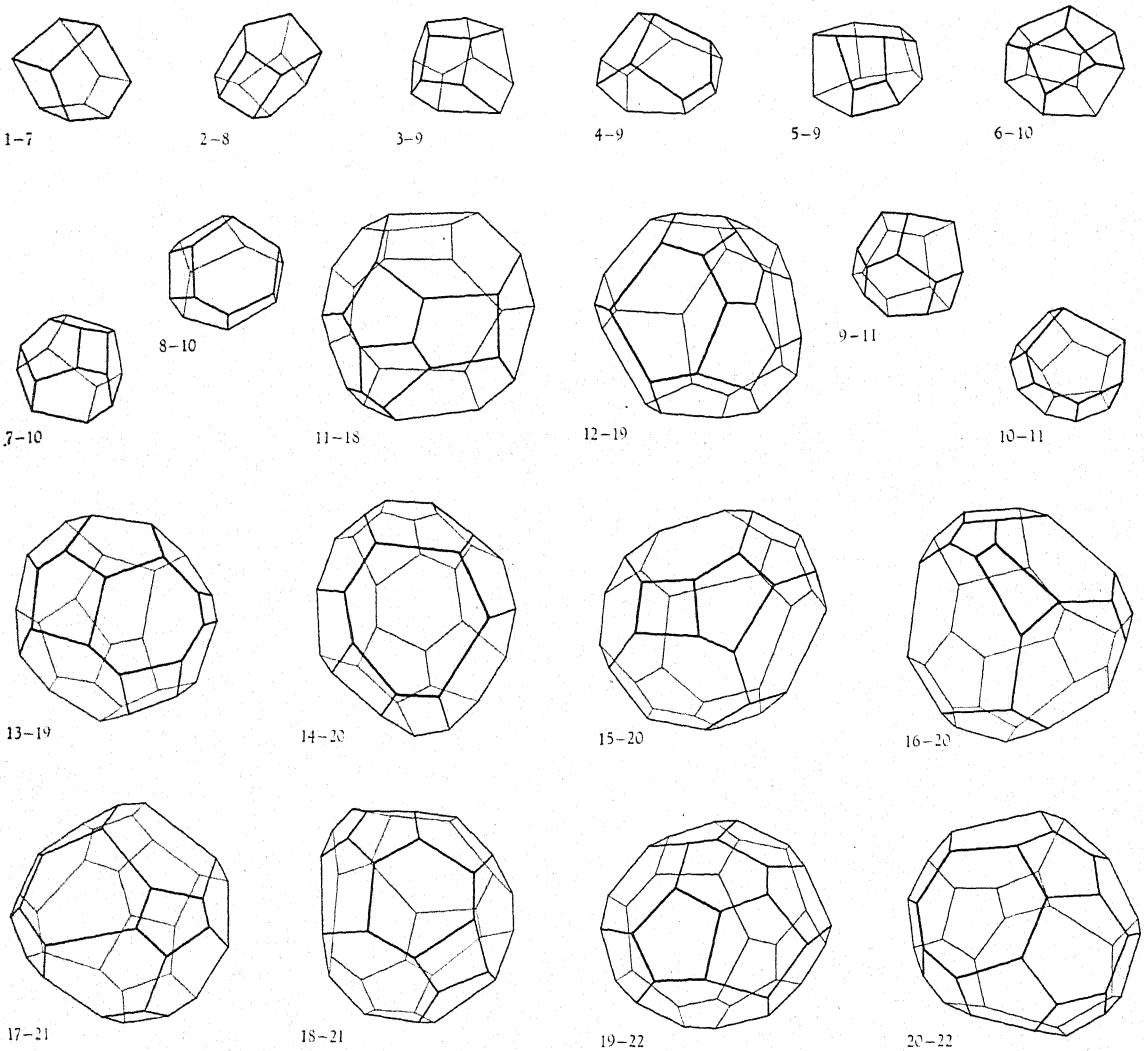


TABLE 9. *Figures 1 to 20. Camera lucida drawings of small and large bubbles from experiment 1. Figures 1 to 10 are of small bubbles (.05 cc.). Figures 11 to 20 are of large bubbles (.4 cc.). × ca. 2.9. The second number under each figure indicates the total number of faces.*

Small bubbles									Large bubbles									
	No. of sides per face			Total	Bubbles found Exp. No.					No. of sides per face					Total	Bubbles found Exp. No.			
Fig.	4	5	6	faces	1	2	3	Total	Fig	4	5	6	7	8	faces	1	2	3	Total
1	5	2	.	7	1	.	.	1	11	1	11	5	1	.	18	1	.	.	1
2	4	4	.	8	4	.	.	4	12	1	10	8	.	.	19	1	.	.	1
3	3	6	.	9	8	1	.	9	13	2	11	3	3	.	19	1	.	.	1
4	4	4	1	9	8	.	.	8	14	1	12	5	2	.	20	2	.	.	2
5	5	2	2	9	1	.	.	1	15	2	10	6	2	.	20	4	.	.	4
6	2	8	.	10	3	.	.	3	16	2	11	4	3	.	20	2	.	.	2
7	3	6	1	10	14	2	.	16	17	1	11	8	1	.	21	1	.	.	1
8	4	4	2	10	1	.	.	1	18	1	12	6	2	.	21	1	.	.	1
9	2	8	1	11	3	8	1	12	19	.	14	6	2	.	22	1	.	.	1
10	3	6	2	11	4	4	.	8	20	1	13	5	3	.	22	2	.	.	2

38, was found three times in experiment 2 and four times in experiment 3. Another 30-faced bubble—0-14-14-2—was the second most common, having occurred five times (fig. 54, table 11). It is not

without significance that the faces on these foam cells were almost all pentagonal and hexagonal. There were 3 large bubble combinations which were found four times: two of them are illustrated—2-10-6-2 (fig. 15, table 9, 20 faces) and 0-14-12-2 (fig. 50, table 11, 28 faces); the third—0-13-15-1 (29 faces)—is not shown in the drawings. In each of these foam cells the overwhelming preponderance of pentagonal and hexagonal faces is evident again. Of the total 105 combinations on the 150 large bubbles, 4 were found three times: 3-10-5-4 (22 faces); 1-12-13-2 (fig. 36, table 10, 28 faces); 1-12-14-2 (fig. 52, table 11, 29 faces); and 1-13-14-3 (fig. 39), table 10, 31 faces). Here again the faces were nearly all pentagonal and hexagonal. There were 18 combinations among the 105 of the large bubbles which occurred twice; 6 of these are illustrated in figures 14, 16, 20, 34, 58, and 59, and their combinations are listed in tables 9, 10, and 11. The remaining 17 illustrations of large bubbles, which are also recorded in tables 9, 10, and 11, occurred just once. Of the 105 combinations, 78 were found only once.

Among the 105 combinations of the 150 large bubbles, there were 2 which had a nonagonal face, and each of them occurred just once; there were 16 combinations with one or more octagonal faces, as in figures 31, 32, and 35, all of which occurred just once; and there were 10 combinations with 4 or 5 quadrilateral faces, all of which were found only a single time. These data further emphasize the fact that the large bubbles most commonly had a preponderance of pentagonal and hexagonal faces.

Large faces of the foam cells, such as the conspicuous heptagonal one in figure 14 and the octagonal one in figure 31, were formed when two large bubbles were in contact, with their centers relatively close together. The great majority of the smaller faces represented contacts between large and small bubbles.

Discussion.—A consideration of tables 1, 2, and 3, and of the curves in figure 61 shows that the number of contacts in the cells of a foam bears a definite relationship to the size and proportionate numbers of the foam cells. It has been demonstrated previously that bubbles of uniform volume in a foam have an average of nearly 14 faces—13.70 for 600 foam cells (Matzke, 1946). When bubbles of varying volumes were used—in this case the volumes bore the ratio of 1:8—the small bubbles had fewer faces than 13.70 and the large ones had a greater number. When the ratio of number of small to large bubbles was 2:1, the average number of contacts dropped in the small bubbles to 9.68, while that of the large bubbles rose to 20.42. When the number of small to large bubbles was 8:1, the number of contacts in the small bubbles rose to 11.90, while that of the large ones increased to 27.34. In the third experiment, in which the number of small to large bubbles was 32:1, the average for the small bubbles was 13.20, while that of the large ones was 30.06. Increasing the proportion of small bubbles obviously brought their average closer and closer to 13.70, as would be expected; increasing the proportion of small bubbles also increased the number of contacts on the large ones. This latter increase would be expected to continue and to get farther and farther from 13.70, until the large bubbles were so infrequent that they were always surrounded completely by small ones. In the experiments here described, the volume ratio of small and large bubbles was kept constant at 1:8. This is within the range of volume variation which Marvin (1939b, 1944) obtained for cells of *Eupatorium* pith, although his average ratio was approximately 1:3 (1944). If the difference in volumes were increased further, still greater numbers of faces would be expected in the larger foam cells, and smaller numbers in the small ones, other factors being constant. Therefore, in a cellular tissue in which the volumes of the cells vary, the range in number of contacts would be expected to be greater than in a tissue in which the cells are of relatively uniform volume. This is brought out in table 12. It can be seen there that 600 bubbles of uniform volume had a range in number of faces from 11 to 17; in 700 undifferentiated cells the range was from 6 to 22; in 300 bubbles of varying volumes the range was from 7 to 33; while in 300 compressed lead shot of similarly varying volumes, the range was from 6 to 36 contacts. The range in number of contacts of the 700 cells is due in part (but only in part) to the size differences.

No one "type" of foam cell was actually found which was characteristic of uniform bubbles; even less would a "type" be expected to occur in bubbles of different sizes. Since plant and animal cells are subject to other factors, and size variations also occur, conformity to one "type" would be even more surprising in such tissues; and no such type has been found, constant in number of faces, in kinds of faces, and in arrangement of faces. Division of space into "ideal" units was described by Lord Kelvin (1887). His "orthic" and "minimal" tetrakaidecahedra may be conceived of as "types," and Lewis has demonstrated possible interrelationships between combinations of faces in plant and animal cells and Kelvin's types. The Kelvin figures, having 8 hexagonal and 6 quadrilateral faces, were not found in assemblages of bubbles of uniform volume (Matzke, 1946), and they were not present in the mixtures of small and large bubbles here described. Nevertheless, the importance of 14 as a basic number of contacts is apparent in all this work.

It is evident from table 12 that the range in number of contacts in bubbles of uniform volume was narrow (from 11 to 17); in the 700 undifferentiated cells tabulated the range was from 6 to 22, while in the mixed bubbles the range was from 7 to 33. In the data on bubbles here presented, the volume ratio of the small to the large was 1:8. For most of the 700 cells recorded in table 12, no volume measurements have been given. However,

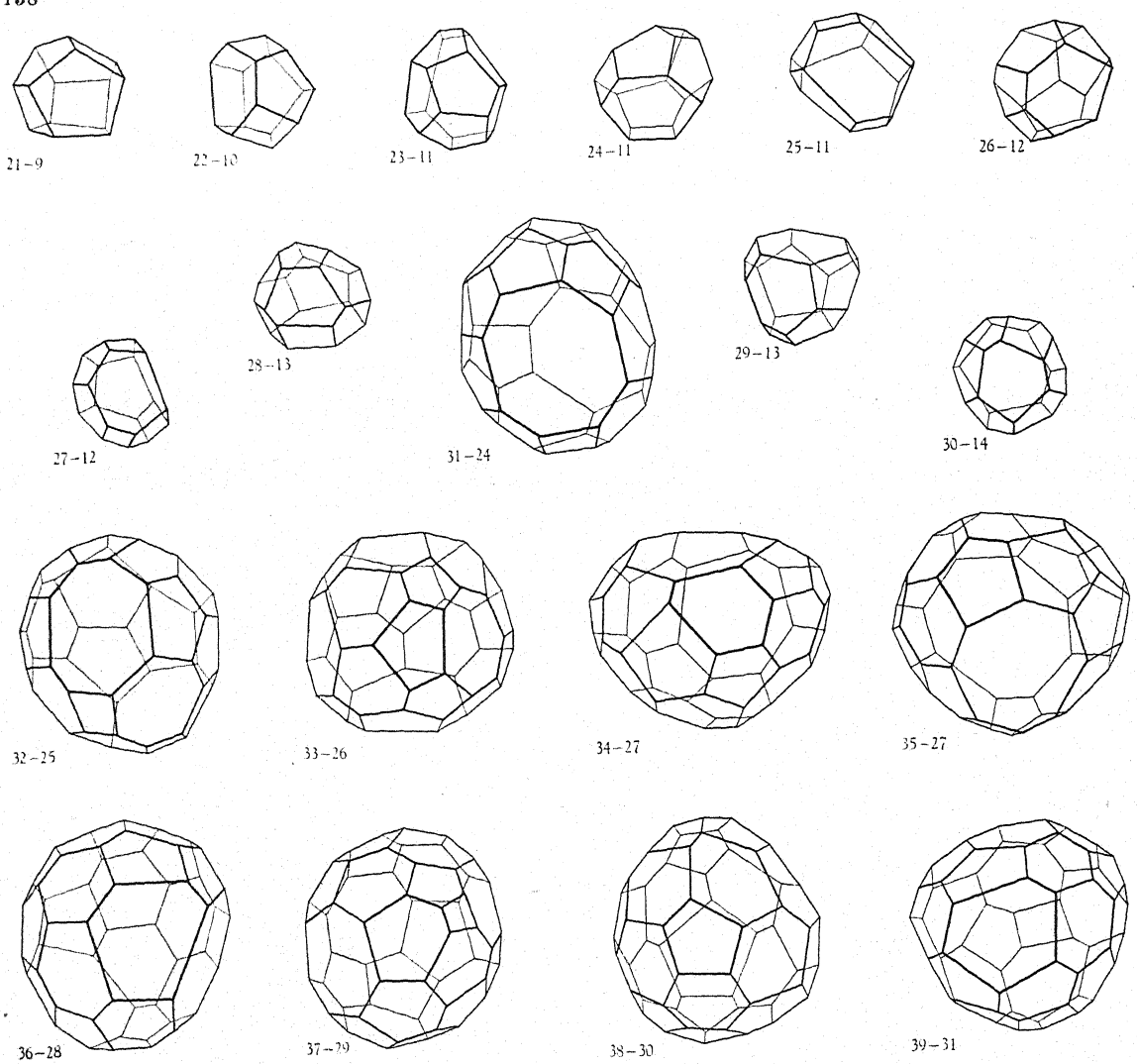


TABLE 10. *Figures 21 to 39. Camera lucida drawings of small and large bubbles from experiment 2. Figures 21 to 30 are of small bubbles (.05 cc.). Figures 31 to 39 are of large bubbles (.4 cc.). × ca. 2.9. The second number under each figure indicates the total number of faces.*

Small bubbles									Large bubbles									
	No. of sides per face			Total	Bubbles found Exp. No.					No. of sides per face					Total	Bubbles found Exp. No.			
Fig.	4	5	6	faces	1	2	3	Total	Fig.	4	5	6	7	8	faces	1	2	3	Total
21	3	6	.	9	8	1	.	9	31	1	13	8	1	1	24	.	1	.	1
22	3	6	1	10	14	2	.	16	32	2	13	6	3	1	25	.	1	.	1
23	2	8	1	11	3	8	1	12	33	.	14	10	2	.	26	.	1	.	1
24	3	6	2	11	4	4	.	8	34	1	13	10	3	.	27	.	2	.	2
25	4	4	3	11	1	1	.	2	35	1	15	8	1	2	27	.	1	.	1
26	2	8	2	12	.	4	6	10	36	1	12	13	2	.	28	.	2	1	3
27	3	6	3	12	1	4	4	9	37	1	14	10	4	.	29	.	1	.	1
28	1	10	2	13	.	5	7	12	38	.	13	16	1	.	30	.	3	4	7
29	3	6	4	13	.	2	4	6	39	1	13	14	3	.	31	.	1	2	3
30	.	12	2	14	.	2	.	2											

Marvin (1944) studied 50 small and 50 large cells of *Eupatorium* pith, with a volume ratio of approximately 1:3, and his range of contacts was from 9 to 20. Presumably, his volume relationships are broadly applicable to the cells in table 12 (in which they are not included). There can be little

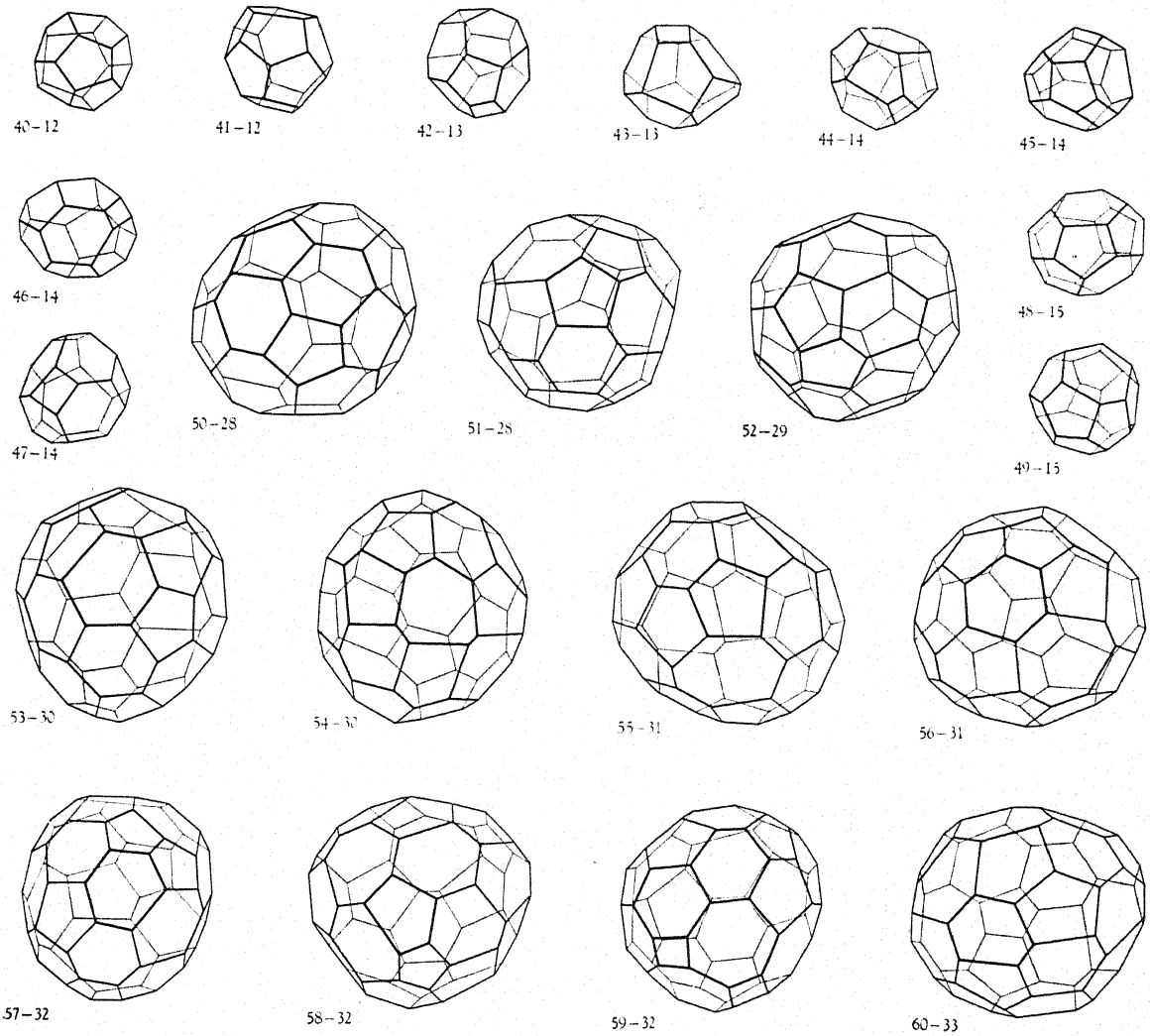


TABLE 11. *Figures 40 to 60. Camera lucida drawings of small and large bubbles from experiment 3. Figures 40 to 49 are of small bubbles (.05 cc.). Figures 50 to 60 are of large bubbles (.4 cc.). × ca. 2.9. The second number under each figure indicates the total number of faces.*

Small bubbles									Large bubbles									
	No. of sides per face				Bubbles found Exp. No.					No. of sides per face						Bubbles found Exp. No.			
Fig.	4	5	6	Total faces	1	2	3	Total	Fig.	4	5	6	7	8	Total faces	1	2	3	Total
40	.	12	.	12	1	4	4	9	50	.	14	12	2	.	28	.	2	2	4
41	2	8	2	12	.	4	6	10	51	3	11	9	5	.	28	.	.	1	1
42	2	8	3	13	.	1	3	4	52	1	12	14	2	.	29	.	.	3	3
43	3	6	4	13	.	2	4	6	53	.	12	18	.	.	30	.	.	1	1
44	1	10	3	14	.	.	1	1	54	.	14	14	2	.	30	.	1	4	5
45	2	8	4	14	.	2	4	6	55	.	15	13	3	.	31	.	.	1	1
46	3	6	5	14	.	.	3	3	56	2	11	15	3	.	31	.	.	1	1
47	4	4	6	14	.	.	1	1	57	.	12	20	.	.	32	.	.	1	1
48	.	12	3	15	.	.	3	3	58	1	13	15	3	.	32	.	.	2	2
49	1	10	4	15	.	.	1	1	59	2	11	16	3	.	32	.	.	2	2
									60	.	14	17	2	.	33	.	.	1	1

question that if the volume ratio had been greater than 1 to 3, the range in the number of faces of the cells would also have been greater. The 700 cells of table 12 include 250 studied by Lewis (1923, 1925, 1933) and 450 undifferentiated cells analyzed in the Laboratory of Plant Morphology at

TABLE 12. *Number of faces*

	Number												
	6	7	8	9	10	11	12	13	14	15	16	17	18
Central bubbles 600	0	0	0	0	0	2	73	179	218	106	20	2	0
Lewis 250 cells	1	1	2	3	7	17	32	42	46	41	30	9	7
Columbia 450 cells	0	1	2	6	28	39	64	67	77	60	47	25	23
Total 700 cells	1	2	4	9	35	56	96	109	123	101	77	34	30
Mixed bubbles 300	0	1	4	18	21	23	36	26	13	6	1	2	5
Mixed shot 300	1	1	7	19	23	15	24	33	17	9	2	2	5

[a] The number below, in parentheses, is the average, including Higinbotham's "lost contacts."
[b] The number below, in parentheses, is the average based on the figures actually given in this table. The number the experiments.

Columbia University (Marvin, 1939b; Higinbotham, 1942; Dodd, 1944; Hulbary, 1944).

The volume ratio of the small to the large bubbles (1:8), and the numbers of small and large bubbles used, paralleled exactly the conditions reported previously in similar experiments in which lead shot of two different sizes mixed in three proportions were compressed to eliminate interstices (Matzke, 1939). The results outlined in tables 1, 2, and 3 above are similar in a general way to those obtained with the lead shot. However, the range in number of contacts was somewhat more limited in the bubbles than in the shot. In table 1, for instance, the range for the small bubbles was from 7 to 12, and for the large bubbles from 17 to 24; in the similar shot experiment the ranges were from 6 to 13 and from 15 to 24.

The kinds of faces—that is, quadrilateral, pentagonal, hexagonal—were also similar in the experiments with bubbles and with shot, but the range was more limited in the bubbles (fig. 62). For example, in experiment 3 the faces varied from 4- to 7-sided in the small bubbles and from 4- to 8-sided in the large ones (table 6). In the parallel lead shot experiment the range was from 3- to 8-sided in the small shot and from 3- to 10-sided in the large shot. The number of pentagonal faces in the small bubbles in the three experiments added together was 1,064, contrasted with 760 pentagonal faces in the small shot. Similarly in the large bubbles there were 1,747 pentagonal and 1,594 hexagonal faces, while in the large shot, in parallel experiments, there were 1,282 pentagonal and 1,188 hexagonal faces. This strong preponderance of pentagonal faces in the bubbles, and to a less extent of hexagonal faces, was not merely fortuitous. A long time ago Plateau (1873) demonstrated that edges in soap films meet at angles of 109°28′16″. The angles of a regular pentagon are 108°—very close to this required angle; the angles of a regular hexagon (120°) are closer to this required angle than the angles of a square (90°). From the standpoint of stability of angles between edges, then, the pentagon comes close to meeting this necessary condition, especially if the sides are curved, as they frequently are, and the hexagon does not require too great distortion. The occurrence of considerable numbers of pentagons in the compressed shot may be ascribed in whole or in part to the geometrical necessities of the aggregation of such polyhedra. The finding of larger numbers of pentagons in the foam cells, and the distinctly greater uniformity and regularity of the latter, may be ascribed to the imposition of surface forces upon the geometrical necessities already present in the compressed lead shot.

The 35 different combinations of faces found in the 150 small bubbles studied in experiments 1, 2, and 3 are listed in table 7. The 150 large bubbles, on the other hand, occurred in 105 different combinations. If these bubbles had all been of equal volume—either large or small—the number of combinations would have been far fewer. As shown in table 13, 600 bubbles of uniform volume occurred in only 36 combinations. Varying the size of the bubbles, therefore, increased the number of combinations. However, 624 central shot, of uniform volume, occurred in 303 combinations, while 300 shot of varying volume were found in 234 combinations. Although the geometrical limitations were not precisely similar in these two types of systems, due to the presence of tetrahedral angles in the shot and their absence in soap film systems, it is, nevertheless, very evident that the number of combinations was much lower in the soap films. This may be ascribed to the relatively greater importance of the surface forces in liquid films.

In table 13 the combinations in bubbles, shot, and cells are listed. It is immediately obvious that the 550 undifferentiated cells recorded there were found in more combinations than were bubbles of uniform volume, and in fewer combinations than lead shot of uniform volume. However, these cells were not of uniform volume. Since varying the volumes increases the number of combinations, as shown above, the cells, which were not at all of uniform volume, would be expected to occur in a larger number of combinations than shot of uniform volume, unless the restricting action of surface forces is of considerable significance. The occurrence of these 550 undiffer-

of bubbles, cells, and shot.

of faces 19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	Average
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.70
9	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.972
7	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.80[a] (13.86)
16	4	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.86[a] (13.90)
6	14	12	10	0	6	7	6	12	13	15	22	13	7	1	0	0	0	13.53[b] (18.77)
14	17	7	4	9	7	8	10	7	15	10	10	11	3	5	3	1	1	13.45[b] (18.44)

above the parentheses is the weighted average, based on the ratio of small to large bubbles and shot used in each of

entiated cells of varying volumes in 203 combinations, therefore, points to the fact that surface forces were effective when these cellular configurations were determined in or near the plant meristems. This does not mean that they were the only or even the most important factors. Furthermore, in living tissues, forces which are entirely absent in such systems as soap films and lead shot may well be operative, and probably are.

An inspection of tables 7 and 8 reveals that certain combinations occurred more commonly than others and that none was completely predominant over all the rest. The most abundant peripheral and central bubbles of uniform volume previously studied showed for the most part the same combinations of faces found in the small bubbles recorded in table 7; of the 400 peripheral bubbles 352 matched the small bubbles, while 525 of 600 central bubbles

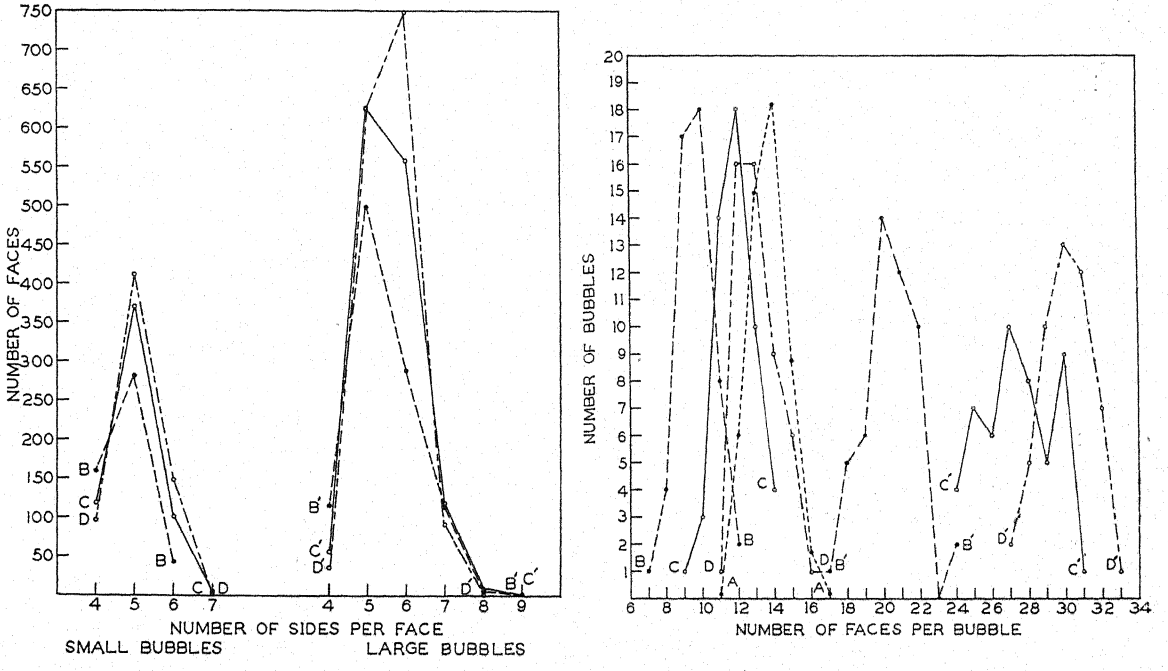


Fig. 61–62.—Fig. 61 (left). Curves showing the number of faces per bubble and the frequency of the occurrence of bubbles with these varying numbers of faces: AA is the curve for bubbles of uniform volumes (Matzke, 1946). BB and B′B′ are curves for the small and large bubbles, respectively, in experiment 1; CC and C′C′ the curves for the small and large bubbles, respectively, in experiment 2; DD and D′D′ the curves for the small and large bubbles, respectively, in experiment 3. BB, CC, and DD get successively closer to AA, while B′B′, C′C′, and D′D′ get successively farther away.—Fig. 62 (right). Curves showing the number of sides per face (4—quadrilateral, 5—pentagonal, 6—hexagonal, etc.) and the frequency of occurrence of each kind of face in small and large bubbles: BB and B′B′ are the curves for the small and large bubbles, respectively, in experiment 1; CC and C′C′ the curves for the small and large bubbles, respectively, in experiment 2; DD and D′D′ the curves for the small and large bubbles, respectively, in experiment 3.

TABLE 13. *Number of combinations of faces in bubbles, shot, and cells.*

	Number of combinations		Number of combinations
300 mixed bubbles		300 mixed shot (Matzke, 1939)	
Experiment 1 50 small bubbles	13	Experiment 1 50 small shot	26
Experiment 2 50 small bubbles	19	Experiment 2 50 small shot	39
Experiment 3 50 small bubbles	21	Experiment 3 50 small shot	41
Total 150 small bubbles	35	Total 150 small shot	88
Experiment 1 50 large bubbles	37	Experiment 1 50 large shot	49
Experiment 2 50 large bubbles	41	Experiment 2 50 large shot	50
Experiment 3 50 large bubbles	33	Experiment 3 50 large shot	50
Total 150 large bubbles	105	Total 150 large shot	147
Total 300 small and large bubbles	140	Total 300 small and large shot	234
600 central bubbles (Matzke, 1946)	36	624 central shot (Marvin, 1939a)	303
400 peripheral bubbles (Matzke, 1946)	43	104 peripheral shot (Marvin, 1939a)	52
		550 undifferentiated cells (various authors[a])	203
		600 Elodea cells (Hulbary, 1944)	31
		Total 1150 cells	214

[a] Dodd, 1944; Higinbotham, 1942; Hulbary, 1944; Marvin, 1939b, 1944.

had combinations similar to those found in the small bubbles.

In tables 7 and 8 it can be seen that the pentagonal dodecahedron, with 12 faces all pentagons, occurred nine times among the 150 small bubbles and was one of the commonly found combinations. This figure comes close to satisfying the Plateau conditions of 120° angles between faces, and angles of 109°28′16″ between edges. In the regular pentagonal dodecahedron angles between faces are 116°33′54″ and the edges form angles of 108°. The regular pentagonal dodecahedron also possesses marked economy of surface to volume (Matzke, 1946). It requires but little distortion, therefore, to fulfil the necessary conditions to a high degree. The finding of these somewhat irregular but easily recognizable dodecahedra is, consequently, very understandable.

In bubbles of uniform volume, as previously demonstrated, 50 of 600 were pentagonal dodecahedra (8⅓ per cent), and this was the fourth most common combination. In the present investigation, none of the large bubbles had fewer than 17 faces, and there were, therefore, no pentagonal dodecahedra. In the small bubbles tabulated above, 9 of the 150 were pentagonal dodecahedra. From table 7 it is obvious that there were 4 pentagonal dodecahedra in experiment 2, and 4 in experiment 3. This constitutes 8 per cent in each of these experiments. In experiment 1 there was only 1 pentagonal dodecahedron, but, as may be seen in table 1, there were only 2 foam cells with 12 faces, and all the rest had fewer contacts. Through curvatures of faces and edges the equilibrium conditions of Plateau and economy of surface to volume seem to be approximated or achieved, even though the regular pentagonal dodecahedron does not occur with overwhelming frequency.

In the case of small lead shot mixed with large and compressed to eliminate interstices in experiments paralleling those of the soap bubbles here described, about half of the small shot matched the 35 combinations shown in table 7 (76 of 150). In the case of peripheral and central compressed shot of uniform volume (Marvin, 1939a), the correspondence was not nearly so great as in the case of peripheral and central bubbles of uniform volume, as shown in table 7. Thus, 57 of 104 peripheral shot and 141 of 624 central shot occurred in the same combinations as the small bubbles. These numbers are obviously smaller than those of the similar experiments on bubbles in which 352 of 400 peripheral bubbles and 525 of 600 central bubbles had the same combinations of faces as the 150 small bubbles (table 7). These results are to be expected, since surface forces are of relatively less effective significance in the compressed shot.

If, now, the combinations of the 550 undifferentiated cells studied by various authors (Marvin, 1939b, 1944; Higinbotham, 1942; Dodd, 1944; Hulbary, 1944) are matched against the 35 combinations in the small bubbles, it is seen that 211 of these cells, or 38 per cent, had the same combinations as these 150 small bubbles (table 7). This is in spite of the fact that the cells occurred in 203 combinations (table 13), while the bubbles were found in 35; in other words, 38 per cent of the 550 cells were distributed in 15 per cent of the total combinations—the 15 per cent which matched the small bubble combinations. This is obviously a fairly close correspondence. There were 192 of these same 550 undifferentiated cells which matched the 36 combinations previously reported for 600 bubbles of uniform volume. The greater number (211) matching the 35 combinations of the 150 small bubbles here recorded is understandable, however, since the cells were not of one volume.

In the corresponding experiments on lead shot, there were 88 combinations among the 150 small shot tabulated, and 251 undifferentiated cells

matched these small shot. Although this is a somewhat larger number of matching cells than in the case of the 150 small bubbles (251 compared with 211), actually there were 43 matching combinations in the small shot, compared with 30 matching combinations in the small bubbles. There were thus 5.8 undifferentiated cells for each of the matching combinations in the small shot, and 7.0 cells for each in the small foam cells.

If the small shot and small bubbles are compared with Hulbary's (1944) 600 more differentiated Elodea cells, it is seen that 180 of the cells matched the small shot (occurring in 88 combinations) and 184 matched the small bubbles (occurring in 35 combinations). Actually there were 11 matching combinations in each.

Furthermore, the cell combination most frequently found among the 550 undifferentiated cells—3-6-4 (fig. 29, 43, tables 7 and 8)—was one of those commonly occurring in the small bubbles. A considerable number of other combinations repeatedly recurring in the cells were also common in these bubbles: 3-6-1 (fig. 7, 22, tables 7 and 8); 2-8-2 (fig. 26, 41); 3-6-3 (fig. 27); and 3-6-2 (fig. 10, 24). A consideration of all these data shows that the small bubbles and cells in tissues bear close resemblance to each other in many ways.

Since the large bubbles were found in 105 different combinations, which did not match previous data very well, these combinations are not being published, though some of them are shown in figures 11 to 20, 31 to 39, and 50 to 60, and the combinations are listed in tables 9, 10, and 11. There were only 8 shot, in 6 different combinations, which matched the 150 large bubbles; there were only 6 undifferentiated cells, in 3 different combinations, which matched those same bubbles. As pointed out above, small and large cells in *Eupatorium* were in approximate volume ratio of 1 to 3 (Marvin, 1944); in the bubbles the volume ratio of small to large was 1 to 8. Since the large bubbles had a high number of faces, close correspondence would hardly be expected in the relatively small number studied (150 in all).

A consideration of the data presented above leads to certain definite conclusions. When bubbles of uniform volume were placed into a container and examined, no "type" was actually present, as previously demonstrated. When bubbles of varying sizes were assembled, as in the experiments here described, many of the same combinations occurred, but conformity to any one pattern or to any group of patterns was even less definite. This does not mean that there was no regularity, since certain foam cells occurred with considerable frequency.

When comparisons were made between bubbles of two different volumes aggregated in varying numbers into a foam, and lead shot of corresponding volumes mixed in similar proportions and compressed to eliminate interstices, it was evident at once that the foam cells exhibited distinctly greater regularity than the compressed shot. This more pronounced constancy in the soap films manifested itself in the number of contacts, in the kinds of contacts—that is, pentagonal, hexagonal, etc.—and in the number of different combinations. Although the geometrical necessities were to a considerable extent similar in both types of systems—soap films and compressed lead shot—the relatively greater importance of the surface forces in the soap films resulted in a reduction in the range of variation in the foam-cell configurations.

It is somewhat difficult to make direct comparisons between bubbles in foam, compressed lead shot, and cells in tissues, since the cells were subject to varying conditions and showed a considerable size range. Nevertheless, the data assembled above indicate strongly that in many ways the cells approximated the bubbles in foam, definitely matching them in number and kinds of faces in many instances. In other respects the plant cells were intermediate between bubbles in foam and compressed lead shot in their three-dimensional shapes, suggesting that surface forces were of relatively less importance in cells than in bubbles in foam, but of greater consequence than in the compressed shot.

The evidence here, as in a previous publication, points unmistakably to the fact that surface forces play a definite rôle in three-dimensional cell shape determination, that they are one of the factors responsible for such shapes, and that they are neither the only factor nor necessarily the most important. There is no evidence here that they are invariably effective in every tissue. Surface forces must be taken into account, however, in evaluating the delicate cellular interrelationships in or near the plant meristems, as a result of which the cellular configurations are established which are of such marked importance in the development of the organism.

SUMMARY

Previous investigation (Matzke, 1946) has shown that bubbles of uniform volume, constructed individually and assembled into a homogeneous foam, have an average of nearly 14 faces (13.70 for 600 interior bubbles).

Using essentially the same technique as before, small bubbles each of .05 cc. volume and large bubbles each of .4 cc. were assembled in three different proportions: in experiment 1 in a ratio of 2 small to 1 large; in experiment 2 in a ratio of 8 to 1; in experiment 3 in a ratio of 32 to 1. In each of these three experiments 50 small bubbles and 50 large ones were examined in the mass by means of a binocular dissecting microscope, and the number and kinds of faces of each were studied and recorded.

In experiment 1 the average number of contacts of the small bubbles was 9.68, and of the large bubbles 20.42; in experiment 2 the averages of small and large bubbles were 11.90 and 27.34, respectively; in experiment 3 the averages were 13.20 and 30.06, respectively. Thus the average number of faces on the small bubbles approached closer and

closer to 13.70 as the relative number of small bubbles was increased, while the average of the large bubbles drew progressively farther and farther away from 13.70 as the number of small bubbles was increased, within the limit of these experiments. The average number of contacts of all central bubbles, large and small taken together, was computed for experiments 1, 2, and 3 and was found to be, respectively, 13.26, 13.62, and 13.71.

When the kinds of faces were tabulated, it was seen that in the small bubbles pentagons were more frequent than all other kinds of faces combined; in the large bubbles pentagons were more abundant than any other kind. The faces were not necessarily plane, nor were the sides of the faces always straight lines.

The 150 small bubbles in the three experiments occurred in 35 different combinations of faces, while the 150 large bubbles were found in 105 combinations. The form that occurred most frequently was a small foam cell with 10 faces—3-6-1—which was found 16 times (fig. 7, 22). In the 150 large bubbles the most frequent combination—0-13-16-1, a 30-faceted foam cell (fig. 38)—was found seven times. In the small bubbles, combinations with large numbers of pentagonal faces were most frequent; in the large bubbles pentagons and hexagons predominated.

The common occurrence of pentagonal faces, and to a less extent of hexagonal faces, as well as the frequency of pentagonal dodecahedra, is to be expected in three-dimensional figures which approximate the equilibrium conditions in soap films outlined by Plateau, namely, 120° angles between films and angles of 109°28′16″ between edges. The pentagonal dodecahedron also achieves economy of surface with reference to volume.

No one "type" was predominantly present in the large or small foam cells in the experiments here described, although certain combinations occurred much more frequently than others.

When the results obtained in these experiments were compared with those of similar lead shot experiments, in which pellets of two different volumes were mixed in varying proportions and compressed to eliminate interstices, it was found that the range in number of contacts, in kinds of faces, and in number of combinations of faces was more restricted in each case in the bubbles than in the similar compressed lead shot experiments.

When data on three-dimensional shapes of cells in tissues were compared with those of bubbles in foam and of compressed lead shot, the cells were similar to the bubbles in foam in definite ways, and they were intermediate between the bubbles in foam and the compressed lead shot in certain respects.

Since surface forces are of considerable importance in the soap films and of much less relative significance in the compressed lead shot, and since the cells resemble the bubbles in foam in some ways and are often intermediate between the bubbles and the compressed lead shot, the conclusion is drawn that surface forces play a part when cell shapes are being determined at or near the plant meristems; they seem to be of less relative importance in cells than in soap films. Surface forces may not always be effective, and they do not necessarily constitute the prime factor in the establishment of cellular configurations. That interfacial forces frequently play a rôle in three-dimensional cell shape determination seems quite definite.

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RESPIRATION AND CELL DIVISION IN PLANTS. I. OXYGEN CONSUMPTION AND CELL DIVISION IN THE LEAVES OF LIGUSTRUM LUCIDUM AND HEDERA HELIX [1]

Alvin V. Beatty

THE PRESENT paper is an attempt to further the knowledge of the relationship of oxygen consumption to cell division with data obtained by use of leaves. Here the number of cell divisions considered is much greater than in fertilized eggs of invertebrates or of algae, which in the past have served as materials for such studies. Some authors have found no relationship, while others have claimed that cell division involves oxidative processes dependent on oxygen consumption.

Lyon (1904) working with *Arbacia,* Warburg (1908) with *Echinus,* Gray (1931) with *Echinus,* Whitaker (1932b) with *Cumingia* and (1933a) with *Chaetopterus,* did not find any relationship between nuclear division and respiration. They concluded that respiration is a function of the cytoplasm and is independent of nuclear syntheses.

Mathews (1907) working with *Echinus,* Vlès (1922) with *Paracentrotus,* Whitaker (1932a, c) with *Fucus* and *Nereis* and (1933a) with *Arbacia,* found a direct relationship between nuclear division and respiration. They concluded that nuclear division increases the rate of oxygen consumption and is, therefore, dependent on oxidative processes in the cell.

More recently, Clowes and Krahl (1937, 1940), Krahl and Clowes (1937, 1940), and Krahl *et al.* (1941) have tested the action of phenols, cyanides, carbon monoxide, and other respiratory inhibitors on cell division using sea urchin eggs. They reported "that fertilized eggs of *Arbacia punctulata* have a small or negligible rate of glycolysis, both aerobic and anaerobic, so that these eggs depend almost exclusively on oxygen consumption for the energy required to support division" (Clowes and Krahl, 1940), and that when respiration was inhibited below a certain value, 30 to 40 per cent of the controls, a reversible block to cell division was produced.

MATERIALS AND METHODS.—Young leaves from five different plants of both *Ligustrum lucidum* and *Hedera helix* were used in this study. Five leaves were collected each hour in both species, using leaves of uniform size from the second pair on a growing shoot; three of these were used for the study of mitotic frequency, two in the respirometer.

The Warburg microrespirometer technic, with modified vessels designed by Pomerat and Zarrow (1937), was used for determination of oxygen consumption. The vessels, including a side-arm chamber for KOH, have a capacity of approximately 30 cc. Although the vessels were larger than necessary, they were found to be convenient for this type of work. The large vessel opening permitted the insertion of a leaf without contamination by the lubricating cement, and the extensive floor allowed the placement of a leaf on a dry surface, with the petiole in the water which remained around the side of the vessel.

Five manometers were used, one of which was the thermobarometer. The experiments were conducted from dusk to daylight and in the dark in order to exclude the process of photosynthesis. The leaves used in the respirometer were weighed, and the volumes were determined by submersion. Following this they were blotted dry and put in the vessels with 1 cc. of distilled water. The leaves were allowed eight to ten minutes for temperature adaptation. The temperature was maintained at 25°C. ± 0.05°.

Readings were taken for fifty-minute periods of each hour, the remaining time being necessary for the adaptation of the new leaves and for the preparation of the vessels for a new run. Although preliminary trials revealed that a leaf could be used for four runs without any great variation in results, due to the time that the leaf has been removed from the plant, each leaf was used for only two runs. This procedure gave hourly readings for two leaves which had been in the vessels one hour and two leaves for two hours.

The procedure used in determining the amount of oxygen consumption is given by Dixon (1943). The data on oxygen consumption are given per gram of wet leaf tissue.

On the leaves used for the study of mitotic activity, an area, between specific veins, was located in the lower half of the leaves for the selection of the material. A millimeter disc of tissue was removed from each leaf and placed in Carnoy's solution (Speese, 1939).

In the preparation of the slides, the tissue was placed in an HCl-alcohol solution for five minutes, or until the tissue became translucent, was transferred back into Carnoy's for two minutes (Baldwin, 1939), and put into aceto-carmine for an hour. After staining, each disc was placed on a slide in a small drop of aceto-carmine, and, by means of a specially constructed apparatus with a plunger, the discs were gently smeared so that they retained their circular shape. The slides were then heated and sealed with a paraffin-gum-acacia mixture.

The number of mitoses was determined by a sampling method. The stages of mitosis used in these experiments were from mid-prophase to mid-telophase. Ten areas were counted and the average ascertained. The smears were then projected and

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the area drawn at a magnification of eight. These projected areas were measured with a planimeter. After derivation of smear area, the total number of divisions per millimeter disc of tissue was calculated, and the average of the three slides was used as the number of divisions for each hour.

Observations.—A survey of more than fifty different species of plants, including herbs, vines, shrubs, and trees, revealed that all plants investigated showed a rhythmic cycle of mitotic activity (unpublished). The cycles are not the same for every species, but the cycle for a given species is the same, except in magnitude, at different seasons of the year.

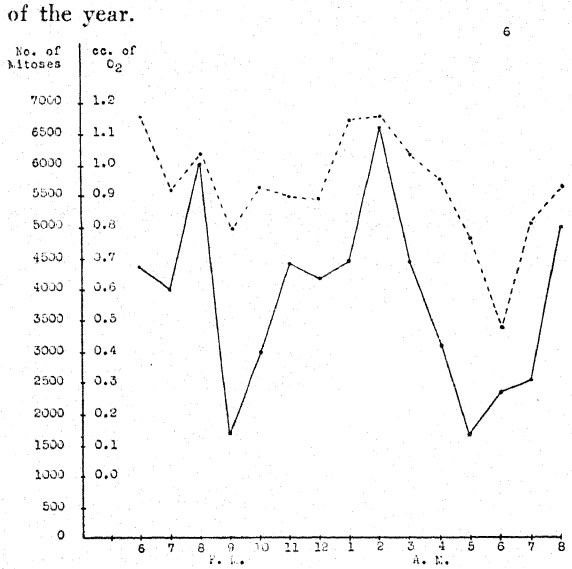


Fig. 1. Number of mitoses per millimeter disc of leaf tissue plotted against time (solid line) and the amount of oxygen consumed per gram of leaf tissue, wet weight, plotted against time (broken line), in *Ligustrum lucidum*.

Ligustrum lucidum was used in this experiment, because in preliminary studies carried out in August, the plant showed a maximum of 11,000 and a minimum of 3,000 divisions per millimeter disc of leaf tissue. During the present investigation, which was carried out in October, there was a maximum of about 6,600 and a minimum of about 1,700. *Hedera helix* was used because the preliminary investigations indicated that the number of divisions was small, about 1,000 as a maximum.

In the present experiments no attempt was made to control the temperature of the plants used for the study of mitotic frequency, since other experiments (unpublished) revealed no correlation between mitotic frequency and temperature for runs continued forty-eight hours. At each hour the leaves were collected from plants growing on the campus of the University of Alabama. The temperature at 6:00 p.m. was 25°C. An hourly drop of about one-half degree in temperature was observed until a low of 17°C. was reached at 7:00 a.m.

In figure 1 (*Ligustrum lucidum*) and in figure 2 (*Hedera helix*) the solid line gives the number of mitoses against time on an hourly basis from 6:00 p.m. to 8:00 a.m., and from 6:00 p.m. to 6:00 a. m., respectively. The character of the curve is typical for most species in that each period of activity is followed by a rapid drop in activity until a low period is reached. Because of the method of calculating the total number of mitoses per hour, the minor fluctuations are not necessarily significant, although they are probably indicative. The present method simply determines the general characteristics and provides a method for comparing periods of high and low activity.

Earlier experiments dealing with oxygen consumption and carbon dioxide production revealed that the amount of oxygen used by the leaves of plants studied (*Ligustrum lucidum, Ligustrum japonicum, Hedera helix,* and *Vinca major*) was not constant during all hours of the night. Although a different curve was found for each species studied, a cursory examination revealed that each species showed at least one period of high, and one period of low oxygen consumption which corresponded in general with the curves for mitotic frequency. The present physiological and cytological experiments were then set up to run concurrently.

In figures 1 and 2 the broken lines give the amount of oxygen consumed per hour per gram of wet leaf tissue against time on an hourly basis. The hourly oxygen consumption shown is an average obtained for the four leaves. The graph for oxygen consumption was superimposed on the graph for mitotic frequency in such a way that there were no intersections.

Since the points on the graphs for both oxygen consumption and mitotic frequency represent averages and do not show individual deviations, table 1 was set up to show the individual variations. This table also provides an opportunity to compare the oxygen consumption of *Hedera helix* and its corresponding low mitotic activity with the oxygen consumption of *Ligustrum lucidum* and its high mitotic activity.

Discussion.—The present paper in no way attempts to analyze the oxidative processes of the cell in relation to cell division as was done by Clowes and Krahl (1941), but rather to present additional information by using plant material to show that there is a relationship between mitosis and oxygen consumption. Clowes and Krahl used only a small number of mitoses. It was the opinion of the author that, if a large number of dividing cells could be studied at one time, the relationship between cell division and oxygen consumption could be more easily observed.

It should be emphasized that the mitotic numbers are based on observations from millimeter discs of leaf tissue while the oxygen consumed is based on a gram of leaf tissue, wet weight, which means approximately forty square centimeters of leaf tissue. In addition, cell division does not take place at an equal rate in all areas of the leaf (Speese, 1941).

TABLE 1. *The hourly variations in oxygen consumption and mitotic frequency in both Ligustrum lucidum and Hedera helix.*

	Ligustrum lucidum		Hedera helix	
	Mitotic frequency	Oxygen consumption	Mitotic frequency	Oxygen consumption
6 p.m.	5078	1.134	57	.467
	3444	.978	92	.654
	4678	1.181	121	.539
	....	1.315	...	.504
7 p.m.	5007	.872	32	.467
	3041	.978	70	.247
	3952	.843	78	.653
	....	.975	...	.428
8 p.m.	5407	.469	75	.914
	6651	1.057	62	.489
	6000	1.387	73	.867
	....	1.242	...	.784
9 p.m.	2718	.595	1100	.743
	822	.828	1512	.784
	1600	.870	268	.590
	....	.853	...	.412
10 p.m.	3422	.677	327	.354
	2692	.938	746	.366
	2901	.918	547	.778
	....	1.159	...	.693
11 p.m.	2727	.853	284	.707
	6194	1.159	809	.630
	4600	.645	557	.359
	....	.923	...	.414
12 m.	5061	1.222	547	.359
	3222	1.492	402	.414
	4300	.115	491	.550
	....	.739	...	.557
1 a.m.	4311	1.243	360	.398
	4362	1.211	300	.504
	4600	1.198	420	.352
	....	.902	...	.352
2 a.m.	6600	.693	603	.352
	6619	2.015	816	.443
	6609	.732	711	.323
	....	1.151	...	.398
3 a.m.	3018	.732	969	.680
	4401	1.393	827	.571
	5800	1.007	904	.431
	....	.998	...	.822
4 a.m.	3572	.604	928	.680
	2667	.713	1256	.675
	3200	1.240	606	.824
	....	1.242	...	.372
5 a.m.	1705	.826	270	.630
	1604	1.056	240	.441
	1800	.555	300	.595
	....	.595	...	.624
6 a.m.	2462	.396	657	.541
	2274	.595	380	.624
	2500	.755	523	.922
	....	.173	...	1.055
7 a.m.	2176	.755	...	....
	2961	.809	...	....
	2700	.933	...	....
	....	.754	...	....
8 a.m.	3301	.933	...	....
	6626	.870	...	....
	5100	.999	...	....
	....	.905	...	....

TABLE 1. *Continued.*

A cursory examination of figures 1 and 2 reveals that there is a correlation between the number of cells in division and the amount of oxygen consumed

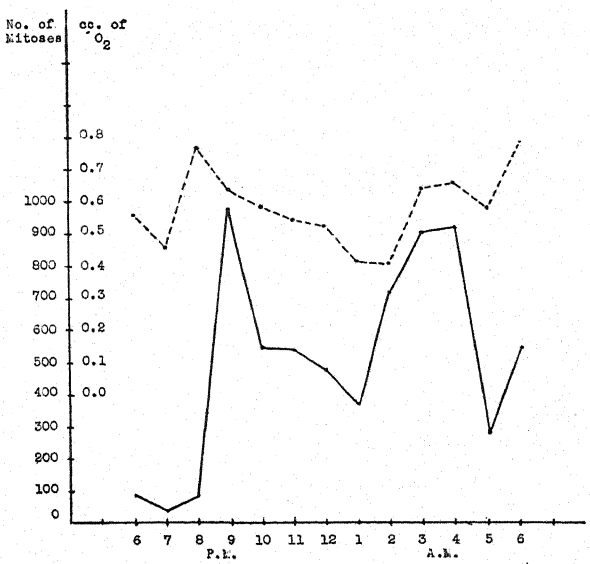


Fig. 2. Number of mitoses per millimeter disc of leaf tissue plotted against time (solid line) and the amount of oxygen consumed per gram of leaf tissue, wet weight, plotted against time (broken line), in *Hedera helix*.

in both plants examined, so that an increase or decrease in the number of cells in division has a corresponding influence on, or is influenced by, the amount of oxygen consumed. The conclusion could, then, be made that a considerable part of the oxygen used by the cells must be used for the process of cell division and subsequent cell enlargement. Clowes and Krahl (1940) found a small or negligible rate of glycolysis in the fertilized eggs of *Arbacia punctulata,* indicating that the eggs depend almost exclusively on oxygen consumption for the energy to support cell division. In order to determine, then, what percentage of the normal oxygen uptake was necessary for cell division, they used low oxygen tension, carbon monoxide, and potassium cyanide. In each case they found that the block to cell division was produced at approximately the same level of oxygen consumption. This was 30 to 40 per cent of that in the controls. Fisher and Henry (1944) found similar results in their experiments with narcotics on cell division in the fertilized eggs of the sea urchin. In 0.1 M urethane, cell divi-

sion was practically stopped, although the rate of oxygen consumption was reduced only about 25 per cent. In studies on growth Commoner and Thimann (1941) and Bonner (1937) working with *Avena* coleoptiles, where practically no cell division is present, found that decreases in respiration decrease elongation. The former authors concluded "that the respiratory requirements of the growth processes cannot represent more than about 10 per cent of the total oxygen consumption."

Fisher and Henry (1944) have assumed two parallel respiratory systems: one, which used about 40 per cent of the oxygen consumed, was called the "activity" system; the other, which used the remaining oxygen, was called the "resting" or "basal" system. Cell division was found to be associated with the "activity" system.

In examining table 1 some evidence can be obtained in support of their conclusions, since the ratio of the oxygen consumed to the number of cells in division in *Hedera helix* is higher than the corresponding ratio in *Ligustrum lucidum*. The "basal" system requires a certain amount of oxygen in each case, below which the oxygen consumption of resting cells would not fall. Since cell division is associated with the "activity" system, which is the variable system, one would expect to find, as one does, a lower ratio in *Ligustrum lucidum* between mitotic frequency and oxygen consumption than in *Hedera helix*.

SUMMARY

Young leaves of *Ligustrum lucidum* and *Hedera helix* were used in this investigation.

The number of mitoses was determined at hourly intervals (6:00 p.m. to 8:00 a.m. for *Ligustrum*, and 6:00 p.m. to 6:00 a.m. for *Hedera*), and graphs were prepared showing mitotic frequency plotted against time. Counts were obtained from three aceto-carmine preparations of millimeter discs of leaf tissue. The sampling method of counting was used.

Oxygen consumption was determined for the same period of time, and graphs were made showing amount of oxygen consumed per gram of wet leaf tissue plotted against time. The values for each hour were obtained by averaging the readings from four leaves.

In each species the graphs showing oxygen consumption and mitotic frequency exhibit common characteristics indicating a correlation between these two processes.

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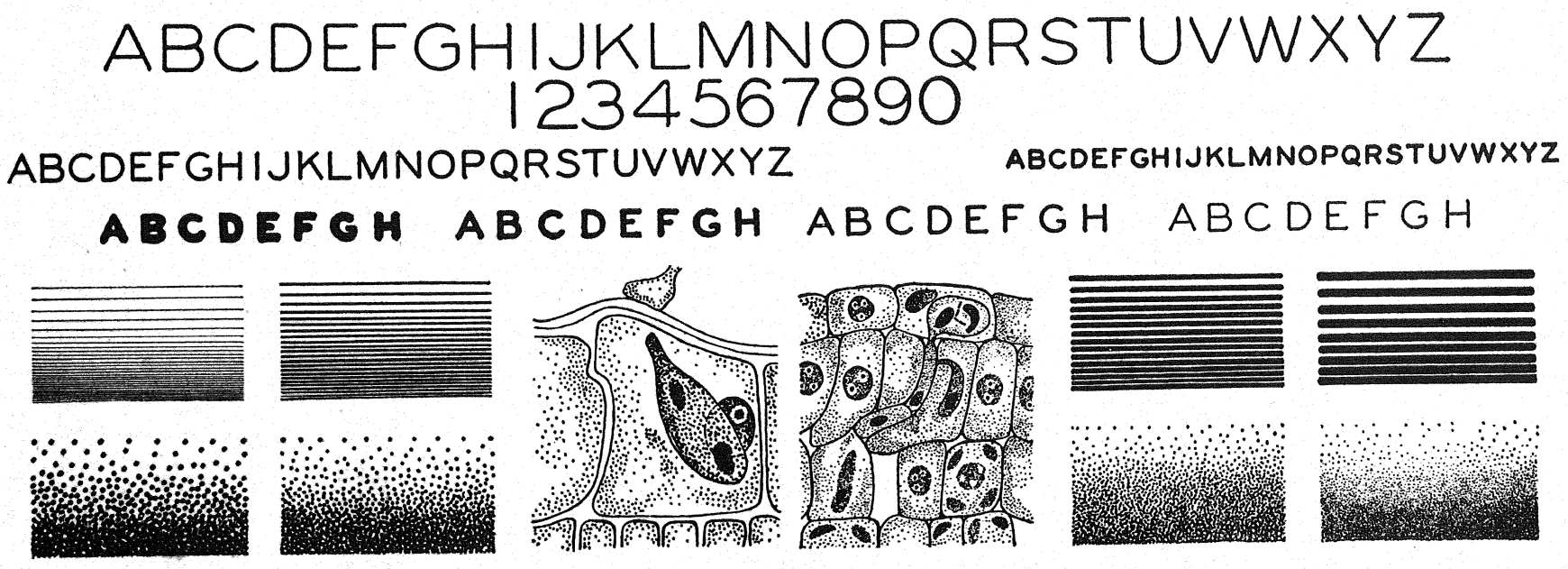
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Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.



INFLUENCE OF THE PROPORTIONS OF KH_2PO_4, $MgSO_4$, AND $NaNO_3$ IN THE NUTRIENT SOLUTION ON THE PRODUCTION OF PENICILLIN IN SUBMERGED CULTURES [1]

Robertson Pratt[2] and Karol A. Hok

INTENSIVE STUDY of cultural conditions that favor biosynthesis of penicillin by different strains of the *Penicillium notatum-chrysogenum* group has been in progress in many laboratories for several years, and it is well known that the strain originally isolated by Flemming in 1929 is not unique in its ability to produce penicillin (Reid, 1933, 1934, 1935; Foster, Woodruff, and McDaniel, 1943; Raper, Alexander, and Coghill, 1944; Raper and Alexander, 1945; Backus, Stauffer and Johnson, 1946).

It was pointed out previously that much of the effort on production has been concerned with the organic substrate that may be used by the mold (see extensive work of Raistrick, Clutterbuck, Lovell, Birkinshaw and co-workers published mainly in the Biochemical Journal since 1932), but relatively little detailed attention has been devoted to the inorganic composition of the culture medium. In this connection it should be observed that in an earlier publication (Pratt, 1945) the important paper of Foster, Woodruff and McDaniel (1943) was inadvertently overlooked. More recently Knight and Frazier (1945) called attention to the importance of the inorganic constituents of corn steep liquor in the biosynthesis of penicillin.

Earlier work in this laboratory (Pratt, 1945) showed that accumulation of penicillin in surface cultures of *Penicillium notatum* can be markedly altered by varying the molecular proportions of KH_2PO_4, $MgSO_4$, and $NaNO_3$ that are added to a corn steep liquor and lactose fermentation medium.

Since current industrial production of penicillin is almost exclusively from submerged cultures, and since the strain of *Penicillium notatum* used in the previous study does not produce well in such cultures, it seemed desirable to determine whether similar results could be obtained with strains of *Penicillium* that are amenable to present commercial methods. This was the object of the experiments reported below.

We believe that the notable differences observed in the response of the several strains of mold to different proportions of salts in the fermentation medium are noteworthy and warrant publication.

The use of corn steep liquor in the solutions introduced certain complications, especially for interpretation of the data. Ideally, the experiments should be repeated in detail, using synthetic media of exactly known composition. However, such a project would have been beyond the scope of our program.

Our primary interest was to ascertain whether higher assays could be achieved in the crude broth by altering the proportions of inorganic salts in the medium, and if so, to determine the optimum relative concentrations of these salts for different strains of *Penicillium* that were deemed of potential commercial importance.

MATERIALS AND METHODS.—Six different strains of *Penicillium,* as shown in table 1, were used in these experiments. Initially, each culture contained 130 ml. of solution in Erlenmeyer flasks of 500 ml. capacity. Flasks were capped with a layer of cotton between two layers of gauze held in place by means of an elastic band. Such caps are considered to permit better aeration of the cultures than do the conventional plugs inserted into the necks of the flasks. After inoculation, the flasks were placed on a shaker and were incubated at 23°C. or at 26°C., as indicated below. Cultures incubated at 23°C. were placed on a shaker (240 oscillations per minute), the amplitude of stroke of which could be adjusted to range from about 6 cm. to 8 cm. As the viscosity of the cultures increased due to growth of the mold, the amplitude was adjusted to maintain as vigorous agitation and aeration of cultures as was possible without causing wetting of the caps. Cultures incubated at 26° were on a shaker that furnished 300 oscillations per minute with an amplitude of 4.5 cm.

The nutrient solution contained lactose, 0.083 M (30 gm./L.); corn steep liquor solids, 20 gm./L.;[3] KH_2PO_4; $MgSO_4 \cdot 7H_2O$ and $NaNO_3$. The combined concentration of the latter three salts was 0.04 M in all solutions, but the proportions of the three were varied. The solutions were adjusted to pH 5.8 to 5.9 by addition of 10 N NaOH before autoclaving. They were autoclaved for 20 minutes at 15 pounds pressure and 118°C. The pH after autoclaving was 5.6 to 5.8.

Inocula for the different cultures were prepared as follows:

1. Eight to ten hours before use 65 ml. sterile tap water were added to profusely sporulating four- to five-day-old cultures on sporulation agar[4] in Erlenmeyer flasks (ca. 225 ml. per 1,000 ml. flask).

[1] Received for publication September 24, 1945.

[2] Currently on leave from the University of California College of Pharmacy.

Acknowledgment is made of the assistance in the laboratory of Ruth Birch, Verna Deromedi, Marjorie Decker, Iola Dunkle, Dawn Hill and Patricia Streator.

[3] Two brands of corn steep liquor, namely, Clinton Company and Corn Products Company's "Argo MVE16," were used in different experiments. The results were similar with both products.

[4] Formula suggested by Dr. A. J. Moyer, Northern Regional Research Laboratory, Peoria, Illinois: NaCl, 5 gm.; $MgSO_4 \cdot 7H_2O$, 0.05 gm.; KH_2PO_4, 0.06 gm.; $Fe_2(CH_4O6)_3$, 0.0149 gm.; $MnSO_4 \cdot 2H_2O$, 0.0034 gm.;

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TABLE 1. *Strains of Penicillium studied on different solutions.*

Species	Isolated at	Derived from	Treatment
P. chrysogenum X1612	Carnegie Institution of Washington, Cold Spring Harbor Station	*P. chrysogenum* NRRL 1951. B25	X-ray
P. chrysogenum 25099	Stanford University	*P. chrysogenum* NRRL 1951. B25	Ultra-violet
P. chrysogenum 35217	Stanford University	*P. chrysogenum* NRRL 1951. B25 through Stanford No. 23248	Ultra-violet
P. chrysogenum 63638	Stanford University	*P. chrysogenum* NRRL 1951. B25 through Stanford Nos. 35217 and 23248	X-ray
P. sp. 1984-A (R-13)	Northern Regional Research Laboratory, Peoria	Organism isolated from soil at the University of Minnesota and designated R-13	This strain (1984-A) represents a selection made at Peoria from cultures of University of Minnesota R-13
P. sp. R-38	University of Minnesota	Organism isolated from soil at the University of Minnesota	None

2. Immediately before use 10 ml. sterile soap solution (0.01 per cent Ivory soap) were added and preparations were shaken to insure uniform distribution of spores.

3. Finally, cultures were inoculated with 1 ml. of above suspension.

The number of spores introduced into each flask of a given series was approximately the same. However, since not all the strains that were used produce (and/or shed) spores equally abundantly, the actual numbers of spores inoculated into the cultures of different strains were not uniform.

Duplicate cultures were inoculated in each experiment and were sampled daily (usually from the third through the tenth day) by withdrawing approximately 0.5 ml. liquor from each culture. The samples were pooled and were assayed in quadruplicate by the standard cylinder plate method employing *Staph. aureus,* NRRL strain No. 313 (F.D.A. strain 209P.) as the test organism. Potencies are expressed in Oxford units. All of the experiments were repeated several times. Averages of the different experiments are presented below.

Plan of experiments.—Table 2 shows the molecular proportions of each of the three principal salts in each of the sixty-five solutions that were tested. The total molarity of these three salts (KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $NaNO_3$) was 0.04 in all solutions in the main series of experiments. This concentration was chosen because it conformed to that of the solution already in use in our laboratories and elsewhere. Figure 1 shows graphically the molecular composition of the sixty-five solutions, each number within the triangle corresponding to a different culture.

$CuSO_4 \cdot 5H_2O$, 0.0041 gm.; peptone, 6 gm.; glycerol, 15 gm.; Brer Rabbit Molasses, 15 gm.; distilled water to make 1 l.; agar, 25 gm.

The complete series of experiments was tested with two strains of mold, namely, X1612 and 1984-A (R-13). Four additional strains were tested on a restricted series consisting of the twenty solutions marked (*) in table 2.

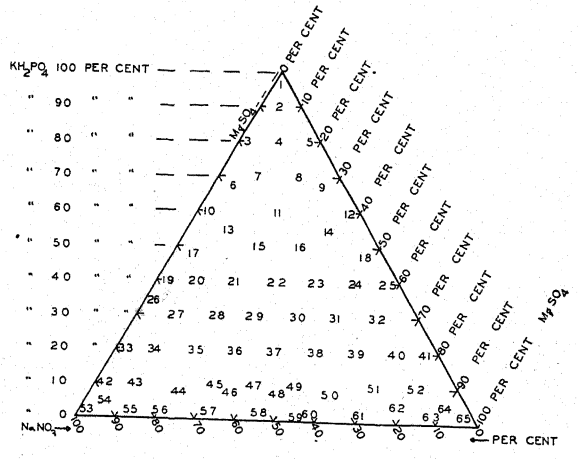


Fig. 1. Chart to show graphically the composition of the different solutions tested. The total molar concentration of the three principal salts in each solution = 0.04. Thus each apex of the triangle represents a concentration of 0.04 M of the particular salt. Reference to this figure facilitates locating the different cultures in figures 2 to 4. Each number within the triangle corresponds to a different culture.

Solutions for the restricted series were chosen to give diverse salt proportions (see location of solutions in figure 1), preference being given to combinations which previous experience indicated might be favorable for increased yields of penicillin.

RESULTS OF EXPERIMENTS.—*Influence of relative concentrations of salts on penicillin production when*

TABLE 2. *Composition of the solutions employed for studying the accumulation of penicillin in submerged cultures of Penicillium chrysogenum. The initial total molarity of the three salts was 0.04 in each solution. The molecular proportions are expressed as percentages of this value. The numbers of the solutions correspond to the numbers within the triangle in figure 1.*

Soln. no.	Molecular proportions[a]			Soln. no.	Molecular proportions[a]			Soln. no.	Molecular proportions[a]			Soln. no.	Molecular proportions[a]		
	KH_2PO_4	$MgSO_4$	$NaNO_3$		KH_2PO_4	$MgSO_4$	$NaNO_3$		KH_2PO_4	$MgSO_4$	$NaNO_3$		KH_2PO_4	$MgSO_4$	$NaNO_3$
1	96.0	2.0	2.0	18	47.5	47.5	5.0	35*	20.0	20.0	60.0	52	10.0	80.0	10.0
2	90.0	5.0	5.0	19	40.0	2.5	57.5	36	20.0	30.0	50.0	53	2.0	2.0	96.0
3	80.0	2.5	17.5	20*	40.0	10.0	50.0	37	20.0	40.0	40.0	54	5.0	5.0	90.0
4	80.0	10.0	10.0	21*	40.0	20.0	40.0	38	20.0	50.0	30.0	55	2.5	12.5	85.0
5*	80.0	17.5	2.5	22*	40.0	30.0	30.0	39*	20.0	60.0	20.0	56	2.5	20.0	77.5
6	67.5	5.0	27.5	23*	40.0	40.0	20.0	40*	20.0	70.0	10.0	57	2.5	32.5	65.0
7	70.0	10.0	20.0	24	40.0	50.0	10.0	41*	20.0	77.5	2.5	58	2.5	45.0	52.5
8	70.0	20.0	10.0	25	40.0	57.5	2.5	42*	9.0	2.5	88.5	59	1.0	55.0	44.0
9	67.5	27.5	5.0	26	35.0	2.5	62.5	43	10.0	10.0	80.0	60	2.5	57.5	40.0
10*	60.0	2.5	37.5	27	30.0	10.0	60.0	44	7.5	22.5	70.0	61	2.5	70.0	27.5
11	60.0	20.0	20.0	28*	30.0	20.0	50.0	45	10.0	30.0	60.0	62	5.0	77.5	17.5
12	60.0	37.5	2.5	29*	30.0	30.0	40.0	46	7.5	35.0	57.5	63	2.5	87.5	10.0
13*	55.0	10.0	35.0	30	30.0	40.0	30.0	47	10.0	40.0	50.0	64	5.0	90.0	5.0
14*	55.0	35.0	10.0	31*	30.0	50.0	20.0	48*	7.5	47.5	45.0	65	2.0	96.0	2.0
15*	50.0	20.0	30.0	32	30.0	60.0	10.0	49	10.0	50.0	40.0				
16	50.0	30.0	20.0	33	20.0	2.5	77.5	50	7.5	60.0	32.5				
17*	47.5	5.0	47.5	34*	20.0	10.0	70.0	51	10.0	70.0	20.0				

[a] Each solution contained, in addition to the three salts shown, 0.083 M lactose and corn steep liquor solids, 20 gm. per liter.
* Solution tested in restricted series.

total concentration of salts remains constant.—1. *General.*—The maximum potencies produced by the several molds in the different solutions and the days on which the maxima were reached are shown in tables 3 to 5. The relative potency in each of the cultures was calculated, using the value for solution No. 42 as one.[5]

Diagrammatic representations of the data for X1612 and for 1984-A are given in figures 2 and 3.

The numbers within the triangles represent the relative concentrations of penicillin in the different cultures, expressed as decimal fractions of that in the standard solution. The short dashes on the sides of the different triangles mark off ten per cent intervals. Each diagram has been divided arbitrarily into three zones, depending upon the concentration of penicil-

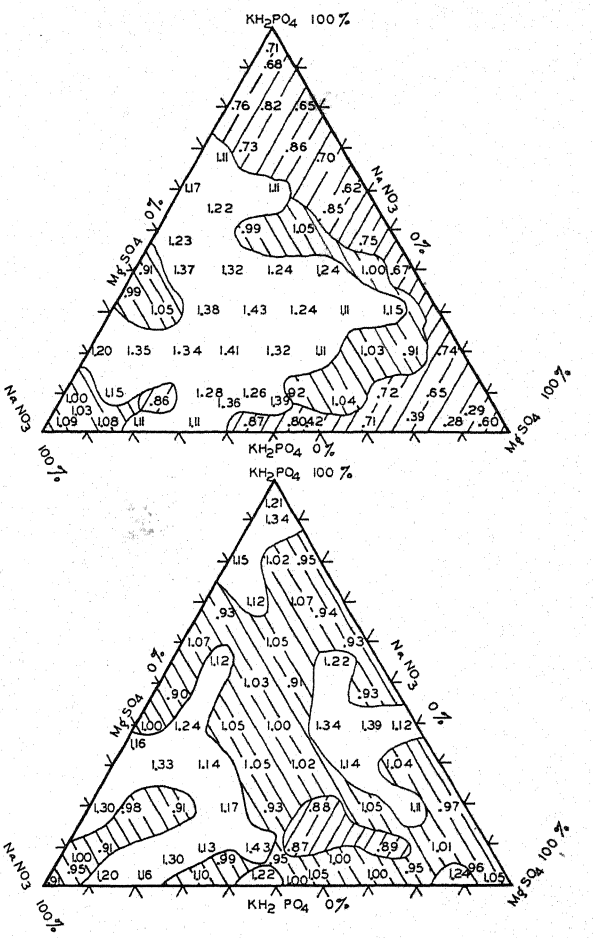


Fig. 2-3 (fig. 2 above, fig. 3 below). Diagrammatic representation of the relative values of maximum potencies obtained in different solutions with X1612 and 1984-A, respectively. The numbers within the triangles represent the maximum potency in the different cultures, expressed as decimal fractions of that in the standard solution (No. 42). The short dashes on the sides of the different triangles mark off 10 per cent intervals.

[5] Solution No. 42 was chosen as the standard because this was the formula that was routinely used in many laboratories.

TABLE 3. *Maximum potency in cultures of Penicillium chrysogenum, strain X1612 on different solutions. (Temperature 26°C.)*

Soln. no.	Max. u/ml.	Days at max.	Relative potency	Soln. no.	Max. u/ml.	Days at max.	Relative potency
1	94	7	0.71	34	180	7	1.35
2	90	8	0.68	35	178	7	1.34
3	101	8	0.76	36	187	7	1.41
4	109	7	0.82	37	176	7	1.32
5	86	7	0.65	38	148	7	1.11
6	146	7	1.10	39	137	7	1.03
7	97	7	0.73	40	121	7	0.91
8	114	7	0.86	41	98	7	0.74
9	93	7	0.70	42	133	7	1.00
10	156	7	1.17	43	153	7	1.15
11	147	8	1.11	44	114	7	0.86
12	82	8	0.62	45	170	7	1.28
13	162	7	1.22	46	181	7	1.36
14	113	7	0.85	47	167	8	1.26
15	131	8	0.99	48	185	7	1.39
16	139	7	1.05	49	124	8	0.92
17	164	7	1.23	50	138	8	1.04
18	100	8	0.75	51	96	8	0.72
19	121	8	0.91	52	86	7	0.65
20	182	7	1.37	53	146	8	1.09
21	175	7	1.32	54	137	7	1.03
22	165	7	1.24	55	144	7	1.08
23	165	7	1.24	56	148	7	1.11
24	133	7	1.00	57	147	7	1.11
25	89	7	0.67	58	116	8	0.87
26	131	7	0.99	59	106	8	0.80
27	139	7	1.05	60	56	7	0.42
28	183	7	1.38	61	94	8	0.71
29	190	7	1.43	62	52	8	0.39
30	165	7	1.24	63	37	8	0.28
31	147	7	1.11	64	39	8	0.29
32	153	7	1.15	65	80	8	0.60
33	160	7	1.20				

lin accumulated in the several cultures at the time of assay. All solutions in which the relative penicillin concentration was between 0.9 and 1.1 are classed together. These are considered "average." Those solutions which were definitely inferior to the standard (relative concentration 0.89 or less) are grouped together and those that seemed superior to the standard (relative concentration 1.11 or greater) have been placed in another group. These different areas are cross hatched in different ways to distinguish one from the other and to facilitate comparison of the two strains of mold. It should be pointed out that the diagrammatic presentation of the data is somewhat subjective, since the contour lines were located merely by inspection. However, although the true picture may differ slightly from the graphic representation, the diagrams are useful for showing trends that are not so readily apparent from examination of the data in tabular form.

2. *Comparison of strains.—X1612 vs. 1984-A.*— Significant differences in response of X1612 and of 1984-A to different molecular proportions of KH_2PO_4, $MgSO_4$, and $NaNO_3$ are suggested by the contours and areas of zones of superior solutions (relative value = 1.11 or more) plotted in figures 2 and 3. Within certain broad limits, changes in the relative concentrations of the three salts were less important for X1612 than for 1984-A. This is indicated by the fact that the area representing superior yields of penicillin was large and relatively continuous, i.e., with few indentations, for X1612, but was smaller and more irregular for 1984-A.

In general, satisfactory yields of penicillin may be obtained with X1612 when the KH_2PO_4 and $MgSO_4.7H_2O$ each constitute from 10 to 40 per cent of the total salt concentration, and the $NaNO_3$ varies from 20 to 80 per cent, the concentration of the latter being reduced as the proportion of KH_2PO_4 and/or of $MgSO_4$ is increased. The area included in this range is shown diagrammatically in figure 4. About 70 per cent of the solutions that showed improved yields fall within this area.

The more pronounced effect of KH_2PO_4 and of $MgSO_4$ in comparison with $NaNO_3$ is shown in figure 5 (solid bars). Bars indicating high yielding solutions are scattered over a wider range of concentrations of $NaNO_3$ than of the other two salts and show no indication of a pronounced peak.

Similar treatment of data for cultures of 1984-A produces a quite different picture (fig. 5, open bars).

TABLE 4. *Maximum potency in cultures of Penicillium sp., strain 1984-A (R-13) on different solutions. (Temperature 26°C.)*

Soln. no.	Max. u/ml.	Days at max.	Relative potency	Soln. no.	Max. u/ml.	Days at max.	Relative potency
1	111	7	1.21	34	90	7	0.98
2	123	7	1.34	35	84	8	0.91
3	106	8	1.15	36	108	7	1.17
4	94	8	1.02	37	85	8	0.93
5	87	8	0.95	38	81	7	0.88
6	85	7	0.93	39	97	7	1.05
7	103	7	1.12	40	102	8	1.11
8	98	7	1.07	41	89	7	0.97
9	86	8	0.94	42	92	7	1.00
10	98	7	1.07	43	84	7	0.91
11	97	7	1.05	44	120	7	1.30
12	85	7	0.93	45	104	7	1.13
13	103	7	1.12	46	91	8	0.99
14	112	7	1.22	47	132	7	1.43
15	95	7	1.03	48	87	8	0.95
16	84	7	0.91	49	80	7	0.87
17	83	8	0.90	50	92	7	1.00
18	85	7	0.93	51	82	7	0.89
19	92	7	1.00	52	93	7	1.01
20	114	8	1.24	53	84	6	0.91
21	97	7	1.05	54	87	7	0.95
22	92	7	1.00	55	110	7	1.20
23	123	7	1.34	56	107	7	1.16
24	128	8	1.39	57	101	7	1.10
25	103	7	1.12	58	112	7	1.22
26	107	7	1.16	59	92	7	1.00
27	122	7	1.33	60	97	7	1.05
28	105	7	1.14	61	92	7	1.00
29	97	7	1.05	62	87	7	0.95
30	94	7	1.02	63	114	7	1.24
31	105	7	1.14	64	88	7	0.96
32	96	8	1.04	65	97	7	1.05
33	120	7	1.30				

TABLE 5. *Maximum potencies in cultures of Penicillium chrysogenum, strains Stanford No. 25099, No. 35217, and No. 63638, and of Penicillium sp. strain Minn. No. R-38 on different solutions (Temperature 23°C.).*

	25099			35217			63638			R-38		
Soln. no.	Max. u/ml.	Day of max.	Rel. value	Max. u/ml.	Day of max.	Rel. value	Max. u/ml.	Day of max.	Rel. value	Max. u/ml.	Day of max.	Rel. value
5	44	11	2.44	40	12	0.67	54	9	0.72	75	11	1.02
10	46	12	2.56	48	11	0.80	66	11	0.88	77	9	1.04
13	40	12	2.22	38	12	0.63	66	12	0.88	58	9	0.79
14	23	11	1.28	40	12	0.67	77	12	1.03	68	7	0.92
15	40	12	2.22	30	11	0.50	83	11	1.11	66	7	0.89
17	40	11	2.22	43	12	0.72	87	11	1.16	59	6	0.80
20	46	12	2.56	35	11	0.58	84	11	1.12	64	6	0.86
21	30	12	1.67	48	11	0.80	83	11	1.11	66	6	0.89
22	26	9	1.45	53	11	0.88	83	11	1.11	63	7	0.85
23	30	11	1.67	42	12	0.70	80	11	1.07	76	9	1.03
28	42	12	2.33	62	12	1.03	88	12	1.18	79	6	1.07
29	48	12	2.68	29	9	0.48	89	12	1.19	67	6	0.90
31	30	11	1.67	33	11	0.55	75	12	1.00	58	9	0.78
34	..	..	...	33	9	0.55	77	11	1.03	96	7	1.30
35	18	9	1.00	28	9	0.47	83	11	1.11	84	7	1.13
39	28	11	1.55	44	12	0.73	83	11	1.11	68	7	0.92
40	26	12	1.45	44	12	0.73	65	11	0.87	74	9	1.00
41	21	9	1.17	58	12	0.97	61	11	0.81	57	6	0.77
42	18	9	1.00	60	11	1.00	75	11	1.00	74	7	1.00
48	29	11	1.61	51	12	0.85	75	11	1.00	60	6	0.81

Although precise formulations are not possible on the basis of these experiments, there is some indication that, in general, for maximum yields of penicillin with this strain of mold, the initial concentration of $MgSO_4 \cdot 7H_2O$ should not exceed 10 per cent of the total salt concentration, and that the initial concentration of KH_2PO_4 should be between 2.5 and 40 per cent of the total.

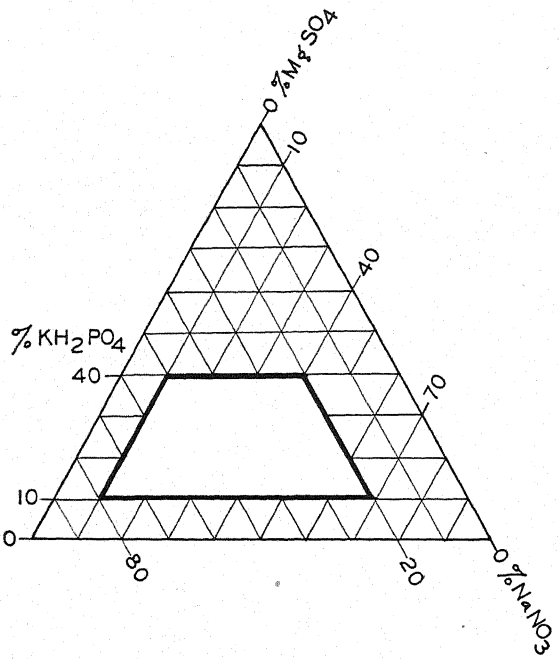


Fig. 4. Diagram to show approximate composition of solutions most suitable to production of penicillin by *P. chrysogenum*, X1612 in shake flasks. (See text for explanation.)

A surprising result of these experiments (fig. 2, 3, 5) is the relatively high yield obtained with both strains of mold on solutions with low partial concentrations of KH_2PO_4 and of $MgSO_4$. This finding is contrary to the behavior of strain 1249.B4 on surface cultures (Pratt, 1945). Another notable feature of the experiments with 1984-A is its performance on solutions with high initial partial concentrations of KH_2PO_4 (fig. 3, 5).

3. *Other strains of Penicillium.*—Table 6 shows the molar compositions of the solutions that produced maximum yields of penicillin with each strain of mold that was studied. The solutions appear to be of four distinct classes, with diverse relative molar concentrations of the salts approximately as follows:

KH_2PO_4 :	$MgSO_4$:	$NaNO_3$	Strain
1 :	1 :	1.3	All strains derived from 1951.B25
1 :	4 :	5	1984-A
1 :	0.5 :	3.5	R-38
1 :	0.1 :	1	1249.B4

We realize that, at best, data obtained in experiments of this type merely represent approximations of the true picture. The differences recorded are of sufficient constancy in successive experiments, however, to suggest that they are real and that they reflect fundamental physiological and biochemical differences among the several strains of fungus.

As was pointed out earlier in connection with similar experiments on surface cultures, the results of experiments such as these are not offered as an ultimate and definitive picture of penicillin accumulation under the conditions of the experiment, but they do serve to focus attention on certain relations that exist between the inorganic composition of the medium and the accumulation of penicillin therein. Although production of penicillin is still of immediate practical concern, the chief permanent value of experiments of this type may reside in the possible significance of the results for fundamental research on metabolism of molds. The data in table 7 are deemed important in this connection, since they afford some measure of the degree of responsiveness of the different molds to relatively simple changes in the inorganic composition of the fermentation medium.

Influence of the total concentration of salts on penicillin production when relative concentrations of the salts remain constant.—All the foregoing experiments were performed at a total salt concentra-

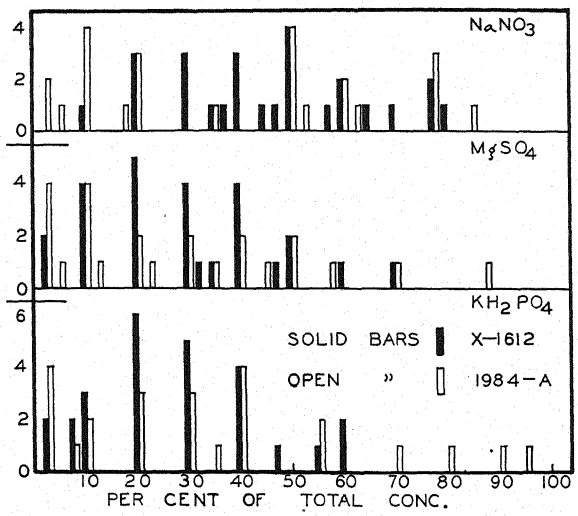


Fig. 5. Histograms to show the number of solutions with each percentage of the different salts that were used in which the maximum potency obtained excelled that in the standard solution (No. 42) by eleven per cent or more. Solid bars are for X1612. Open bars are for 1984-A.

tion corresponding to that of the solution that was currently in wide use, i.e., 0.04 M.[6]

To determine whether the production of penicillin could be increased further by alteration of the total salt concentration, a series of experiments was set up in which only the total concentration of the salts

[6] This is in addition to the salts contributed by the corn steep liquor.

TABLE 6. *Solutions that furnished maximum yields of penicillin with different strains of Penicillium chrysogenum and of P. sp.*

Strain no.	Max.[a] u/ml.	Day of max.	Soln. no.	Millimoles/L. KH_2PO_4	$MgSO_4 \cdot 7H_2O$	$NaNO_3$
X1612	190 (133)	7	29	12	12	16
25099	48 (18)	12	29	12	12	16
35217	62 (60)	12	28	12	8	20
63638	89 (75)	12	29	12	12	16
1984-A (R-13)	132 (92)	7	47	4	16	20
R-38	96 (74)	7	34	8	4	28
1249.B4[b]	..	..	17	19	2	19
Soln. No. 42 contains		..	..	4	1	36

[a] The figures in parentheses in this column show the maximum potency obtained in the standard solution, No. 42, for each strain of mold.
[b] Surface cultures of *Penicillium notatum* (data from Pratt, 1945). This strain is a variant of *P. notatum,* NRRL 1249.B21.

was varied, the relative concentrations remaining constant.

Seven solutions that showed increased yields with X1612 were chosen for this experiment and were compared with the standard, no. 42. Solutions with the molecular proportions shown in table 1 and figure 1 for nos. 17, 20, 28, 29, 36, 42, 46, and 48 were prepared with total concentrations (of these three salts) of 0.02 M, 0.04 M, 0.08 M, and 0.16 M, and were inoculated with X1612.

The relative value of the different solutions for biosynthesis of penicillin did not change significantly from one series to the next. In general best yields were obtained at a total concentration of 0.16 M in a series provided with the standard concentrations of corn steep solids (2 per cent) and of lactose (3 per cent). When corn steep solids and lactose were 3 per cent and 2.5 per cent, respectively, or were 3.5 per cent and 3 per cent, respectively, best yields were obtained, in general, at a total salt concentration of 0.08 M.

SUMMARY

A study was made of the accumulation of penicillin in cultures of different strains of *Penicillium* grown in sixty-five different nutrient solutions containing KH_2PO_4, $MgSO_4 \cdot 7H_2O$, and $NaNO_3$ in different proportions but all with a total molar concentration (of these three salts) of 0.04 M. The solutions contained, in addition, lactose and corn steep

TABLE 7. *Number of solutions tested in which maximum yields deviated from the control by 11 per cent or more—i.e., relative values <0.9 or >1.1.*

Strain	No. of solns. tested	No. solns. deviating from std. 11% or more: Decrease (Rel. val. <0.9) No.	%	Increase (Rel. val. >1.1) No.	%	No change from std. (Rel. val. 0.9–1.1) No.	%	Total per cent of solns. tested that showed significant change in yield	Total range of relative values
X1612	65	24	37	26	40	15	23	77	0.28–1.43
25099	19	0	0	17	90	2	10	90	1.00–2.68
35217	20	17	85	0	0	3	15	85	0.47–1.03
63638	20	5	25	7	35	8	40	60	0.72–1.24
1984-A (R-13)	65	3	5	24	37	38	58	42	0.87–1.43
R-38	20	9	45	1	5	10	50	50	0.77–1.13
1249.B4 (Surface cultures)[a]	65	23	35	11	17	31	48	52	0.62–1.64

[a] Data from Pratt, 1945.

liquor. The results emphasize the importance of obtaining a proper balance among the concentrations of KH_2PO_4, $MgSO_4$, and $NaNO_3$ in the solutions, in order to obtain maximum yields of penicillin. Pronounced differences in the responsiveness of several strains of mold to changes in the relative concentrations of the three salts were observed.

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FURTHER EXPERIMENTS ON THE CULTURE OF EXCISED ASPARAGUS STEM TIPS IN VITRO [1]

Shih-Wei Loo

The writer has shown in a previous paper (Loo, 1945a) that the growth of excised asparagus stem tips (*Asparagus officinalis* Linn.) is potentially unlimited. These excised stem tips were maintained *in vitro* in a synthetic medium under light for a period of nine months and remained as stems without the formation of roots. Evidence has also been presented showing that there are probably two groups of substances which promote the growth of excised asparagus stem tips. One group of these substances is synthesized in the stem tip in the light and is essential for the growth of the stems. The other is apparently manufactured in the root system and accelerates, but may not be essential to stem growth.

Further experiments on unlimited growth, factors affecting growth and histological studies of the excised asparagus stem tips, will be presented in this report. The materials and technique employed have been described in detail in previous papers (Loo, 1945a, 1945b).

Unlimited growth.—Two series of cultures of excised asparagus stem tips have been kept growing *in vitro* through successive transfers for periods of 679 and 260 days, respectively. As the number of transfers progressed, a few changes in the supplementary substances of the culture medium and in cultural conditions were made. The results are summarized in table 1 and 2. Figure 1 is a photograph showing the growth characteristics of two of these stem tips.

The cultures were made at first by using liquid medium in test tubes. Each stem tip was supported by means of a small wad of pyrex glass wool so that only the lower end of the excised stem dipped into the nutrient solution. The inconvenience of this arrangement was later avoided when it was found that the tips could be supported on 0.5 per cent bacto-agar. All the stem tips of the first series were transferred to agar medium at the time of the 27th transfer. Agar medium was used exclusively in the second series of cultures. The results of these two series of experiments are fully comparable. Agar medium of 0.5 per cent was found to be as good, if not better, than liquid medium and in addition provided a simple method for supporting the stem tips.

The data from these experiments show that asparagus stem tips can be maintained through more than thirty-five successive transfers covering a period of more than twenty-two months. It is highly probably that the growth of the excised asparagus stem tips can be maintained indefinitely, since from two months after the beginning of the first culture the growth rate has remained constant in successive transfers.

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GROWTH INFLUENCED BY CLADOPHYLLS.—As pointed out in the previous paper, some of the excised stems *in vitro* produced fewer cladophylls than normal plants. This condition was especially evident when stems were transferred at short intervals of time. This is due either to an after-effect of the wound caused by cutting or to the nature of cladophyll growth, since the tips must grow to a certain length before cladophyll expansion can occur. Microscopic examination of the apex showed the presence of many cladophylls and leaf-scale primordia. Figures 3 and 5 show these primordia. The latter explanation seems more probable since cultures kept in light for long periods of time produced as many cladophylls as normal plants.

During the course of the experiment, it was observed that the growth rate of the stem tips appeared to depend on the number of cladophylls. Consequently, starting at the twenty-sixth transfer of the first series, subcultures were made in which some of the stem tips were cut off beneath the node so that a cluster of cladophylls remained on the tips as shown in figure 1. To illustrate this effect, the growth rate of five such stem tips compared with stem tips cultured by cutting the tips above the node are given in table 3. The data show an average growth rate of about 1 mm. per day for those tips cut below the node in comparison with those cut above the node which grew only about one quarter as rapidly. Two of these typical stem tips are shown in figure 1.

TABLE 1. *Growth of excised stem tips of asparagus in successive transfers in light (Exp. A–1–1).*

Transfer number	Length of transfer interval, days	Number of stem tips	Average growth in mm.	Average growth in mm. per day	Nutrients and cultural conditions
1	7	7	...	...	Basal medium + 2 per cent sucrose; 0.1 mg. per liter thiamine, pyridoxine, under diffuse light
2	15	7	10.9	0.73	Same
3	5	7	10.0	2.00	Same
4	9	7	9.2	1.02	Same
5	7	6	8.7	1.24	Same
6	10	6	9.3	0.93	Same
7	11	7	4.6	0.42	Same
8	10	8	5.2	0.52	Same
9	22	11	11.0	0.50	Same
10	12	11	3.7	0.31	Same
11	16	11	1.4	0.08	Same, but under continuous light and 3 per cent sucrose
12	20	11	1.6	0.08	Same
13	23	11	1.9	0.08	Same
14	11	11	1.1	0.10	Same
15	19	15	6.1	0.32	Same, but under diffuse light and 2 per cent sucrose
16	15	15	3.2	0.21	Same
17	15	14	6.5	0.43	Same
18	9	14	3.8	0.42	Same
19	20	14	11.7	0.58	BM + 2 per cent sucrose, 25 mgs. succinic acid, 100 mgs. $(NH_4)2SO_4$ per liter
20	20	15	6.2	0.31	Same, but 1 per cent sucrose
21	20	15	5.1	0.25	BM + minor elements, 1 per cent sucrose, 0.1 gm. per liter of yeast extract, under diffuse light
22	20	15	7.3	0.36	Same
23	32	18	11.1	0.35	Same
24	31	18	9.4	0.30	Same
25	20	24	7.2	0.36	Same
26	20	26	8.1	0.40	Same
27	19	29	7.6	0.40	Same, but in 0.5 per cent agar medium
28	28	33	10.3	0.37	Same
29	31	38	9.3	0.30	Same
30	31	50	12.4	0.41	Same
31	30	52	11.4	0.38	Same
32	31	55	15.8	0.51	Same
33	30	55	13.2	0.44	Same
34	29	61	9.3	0.32	Same
35	31	64	14.0	0.45	Same

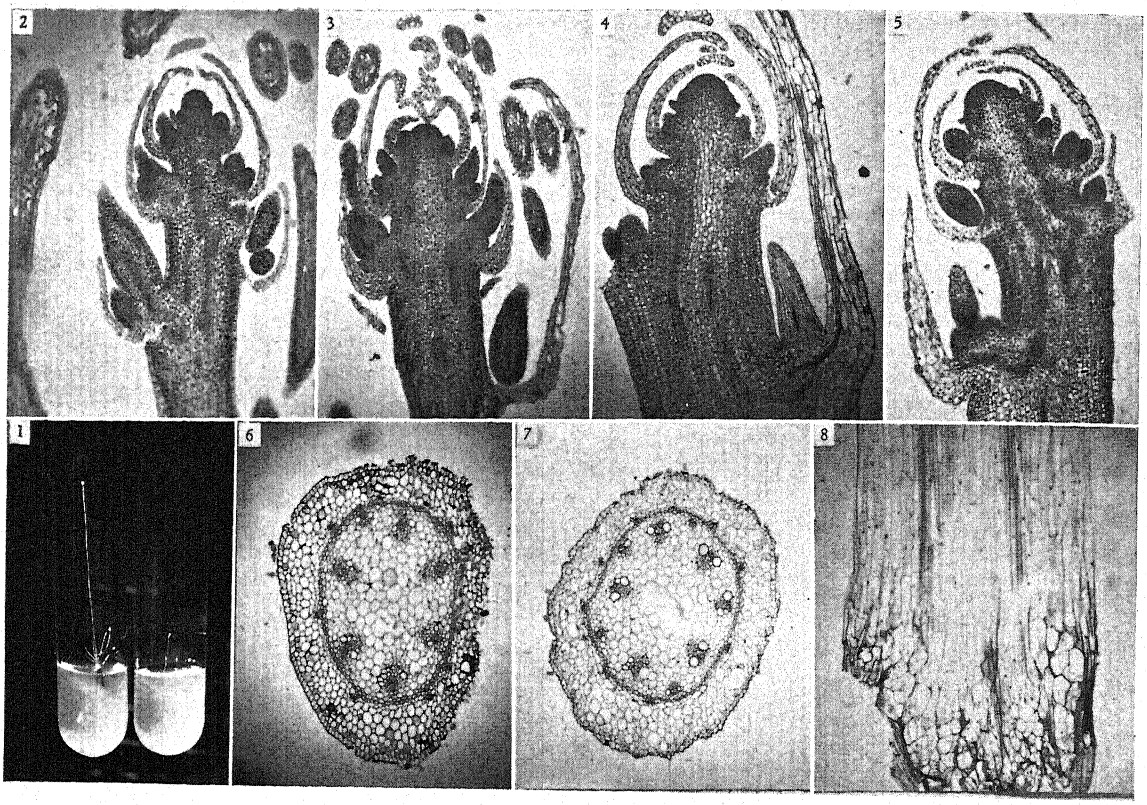


Fig. 1–8.—Fig. 1. Effect of cladophylls on the growth of excised stem tips of asparagus. Subcultures were made by cutting the tips below the node bearing the cladophylls (left) and above the node (right) (photograph, ⅓ ×, exp. A–1–1–3).—Fig. 2–5. Longitudinal sections of the growing point of *Asparagus officinalis* (microphotographs 50 ×).—Fig. 2. Stem tip of intact normal plant.—Fig. 3. Excised stem tip *in vitro* in light in second transfer.—Fig. 4. Excised stem tip *in vitro* in dark in second transfer.—Fig. 5. Excised stem tip *in vitro* in light in twenty-first transfer.—Fig. 6–7. Transverse section of excised asparagus stem tips *in vitro* in light (fig. 6) and in dark (fig. 7) in second transfer (microphotographs 50 ×).—Fig. 8. Longitudinal section of the base of excised asparagus stem tip *in vitro* in light (microphotographs 50 ×).

Clones of excised stem tips.—The possibility of establishing clones by subculture from the lateral buds which developed occasionally on the cultured stems was pointed out in the preceding report (Loo, 1945). At that time, no clone of more than three tips was obtained. At present, the number of stem tips in the original culture series has been increased considerably. Five clones have already been established as shown in table 4. The largest clone has 16 stem tips including the original main tip and the number is still being increased.

Histological studies.—It has been mentioned previously that microscopic examination of fresh materials disclosed no significant difference between excised stem tips and the normal plant. To study the anatomical structures more thoroughly sections were made as follows. Stem tips were killed and fixed in formalin-acetic acid-alcohol solution. They were then dehydrated and embedded in paraffin. Sections were cut serially 8 μ in thickness, in both longitudinal and transverse planes. A modified iron-alum-hematoxylin staining method was used. The material was mordanted in the usual way with iron-alum, but was stained with a very dilute solution of hematoxylin and counter-stained with safranin. All the sections were studied microscopically. Figures 2 to 8 are microphotographs of typical stem tip structures.

The growing points of intact plants and of excised stem tips grown in the light are very similar as shown by longitudinal section in figures 2, 3 and 5. The excised stem tips have as many cladophyll primordia as those of the intact plant even after 21 subcultures (fig. 5). On the other hand, excised stem tips growing in the dark are different from those grown in the light, as shown in figure 4. They have relatively long internodes and rudimentary cladophyll primordia. The terminal portion of the stem tip in the dark is larger in diameter than that of stem tips grown in light. Except for this slight difference, the etiolated stem tip is apparently normal.

Figures 6 and 7 show transverse sections of the differentiated tissues of excised stems growing in light and in the dark. However, the size of the vessels is different; those grown in the dark contain

much larger vessels and have a more irregular epidermis than those grown in the light.

The base of the excised stem tip is generally swollen. Figure 8 is a longitudinal section through the base of a 18-day old, 65 mm. long stem tip. It is apparent that proliferation of cells and callus formation has occurred. However, there is no indication of the formation of root initials in the base of the excised stems. The parenchymatous cells of the basal swelling would appear to be the tissue responsible for absorption of nutrients from the medium.

Discussion.—In general, the growth of the excised stem is quite normal as compared to plants in the field. They form chloroplasts throughout the whole plant body and produce cladophylls in fascicles on each node. Their histological structure is also similar to that of normal plants. The only exception is that the formation of floral organs in asparagus has never been observed on excised stem tips.

Since the functions of the leaf of asparagus are taken over by cladophylls, it is not surprising that the growth of the excised stem tips is greatly affected by the presence of cladophylls as shown in figure 1 and table 3.

Table 2. *Growth of excised stem tips of asparagus in successive transfers in diffuse light at 26°C. in agar medium (Exp. A–26–1).*

Transfer number	Length of transfer interval, days	Average growth in mm. (15 tips)	Average growth in mm. per day
1	20	31.5	1.57
2	20	28	1.40
3	34	26	0.76
4	34	26	0.76
5	34	20	0.59
6	25	13	0.52
7	28	12.3	0.44
8	34	17.2	0.50
9	31	18	0.58

Table 3. *Effect of cladophylls on the growth of excised stem tips of asparagus in successive transfers in basic medium + 1 per cent sucrose, 0.1 gm. per liter of yeast extract, and minor elements in diffuse light at 26°C. (Exp. A–1–2).*

Transfer number	Length of transfer interval, days	Average growth in mm. per day when subcultures were made by cutting the tips	
		below the node bearing the cladophylls	above the node
26	20	1.1	0.25
27	19	1.5	0.20
28	28	0.8	0.30
29	31	0.7	0.24
30	31	0.9	0.28

Table 4. *Number of tips developed in each of the five clones at the time of the 30th subculture.*

Clone number	Number of tips	Mean growth rate in mm. per day[a]
1	16	0.32
2	6	0.39
3	15	0.37
4	5	0.45
5	8	0.47

[a] No differences significant at the 5 per cent level.

Since the potentiality of indefinite growth of excised asparagus stem tips reported previously is further confirmed. These excised stem tips were kept growing *in vitro* for a period of twenty-two months through thirty-five successive transfers.

Dilute agar medium of 0.5 per cent was found to be as good, if not better, than liquid medium and in addition provided a simple method for supporting the stem tips.

The appearance of cladophylls on the stem tips greatly accelerated growth.

Five clones of excised stem tips have been established. The largest clone consists of sixteen stem tips and the number is still increasing.

Histological studies show no significant difference between excised stem tips grown in the light and the normal plant.

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SUMMARY

The potentiality of indefinite growth of excised asparagus stem tips reported previously is further confirmed. These excised stem tips were kept growing *in vitro* for a period of twenty-two months through thirty-five successive transfers.

Dilute agar medium of 0.5 per cent was found to be as good, if not better, than liquid medium and in addition provided a simple method for supporting the stem tips.

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Histological studies show no significant difference between excised stem tips grown in the light and the normal plant.

AUXIN–PROTEIN COMPLEXES OF THE WHEAT GRAIN [1]

S. A. Gordon

It is now generally accepted that auxins occur in plants in other than a "free" or readily extractable form. It is likewise becoming clear that the "bound" forms, in part, are constituents of protein complexes. Skoog and Thimann (1940), by digesting *Lemna* fronds with proteolytic enzymes, were able to secure increased yields of auxin. They subsequently elaborated upon and extended these experiments to tobacco leaves with similar results (Thimann, Skoog, and Byer, 1942). Wildman and Gordon (1942) were able to show that auxin is associated with proteins isolated from the leaves of spinach. This auxin was released by *in vitro* enzymatic hydrolysis. Thus the postulate that leaf auxin exists, in part, naturally bound in an auxin-protein complex (Skoog and Thimann, 1940; Thimann and Skoog, 1940) was substantiated. Whether the same type of association exists in other plant organs, such as the seed, was not known.

Avery, Berger, and Shalucha (1942), treating wheat grains by alkaline hydrolysis, found no apparent relation between the auxin yield and protein content of several varieties of wheat. They obtained relatively large amounts of auxin, as did Haagen-Smit, Leech, and Bergren (1941, 1942), by treatments at an alkaline pH level. These workers attribute the large yields of auxin obtained to the conversion of an auxin precursor. Haagen-Smit *et al.* (1942) were able to secure a small increase in auxin yield when they incubated wheat with chymotrypsin, but attributed the increase to the alkaline medium used. It was considered by Thimann *et al.* (1942) that the bound forms of auxin in *Lemna* were unlike those of the corn endosperm, since from the former material no auxin could be liberated either by extraction with water or by alkaline autoclaving. Gordon and Wildman (1943), however, demonstrated that comparatively large amounts of auxin were released when the cytoplasmic proteins of spinach leaves were hydrolyzed in alkali. Further work was done by Avery and coworkers (Avery, Berger, and Shalucha, 1941; Berger and Avery, 1944a, b) on the properties of a corn endosperm precursor from which indoleacetic acid was obtained by alkaline hydrolysis. Infra-red absorption spectra, nondialyzability, and a partial conversion by proteolytic enzymes of their precursor suggested that it either was a protein or was associated with a protein; this was contraindicated, however, by its low nitrogen content and absence of sulfur. In view of the above research, further investigation of auxin-protein relationships was considered pertinent and desirable.

For a study of seed protein-auxin relationships, the wheat grain was deemed well suited. This seed has been the subject of previous auxin research and its proteins have been extensively investigated. If auxin is associated with the protein of the grain, various relevant questions arise. Is auxin combined with one protein or with more than one? If found with more than one protein, what is the relative apportionment of the auxin, and are these auxin-proteins restricted to the embryo or endosperm? Are the auxins of the different proteins similar? How is auxin associated with protein? Governed by these considerations, the subsequent study was performed.

Experimental outline.—*Preliminary experiments.*—First investigated was the probability that auxin-protein complexes exist in the grain. Flaked wheat germ[2] (embryos) and endosperm flour[2] were treated with live steam for ten minutes to inactivate their contained enzymes. The meals were dried at 60°C. and ground to pass 100-mesh screens. They were then extracted for three days in a Soxhlet apparatus with purified ethyl ether. Both germ and endosperm powders were then digested by a tryptic preparation following the methods previously used by Wildman and Gordon (1942). Auxin concentrations in the enzymatic digests of both meals were roughly three or four times greater than those obtained from parallel controls. (The ratio for the germ was 830:200, and for the endosperm 370:140 micrograms/kg. auxin equivalents.) The existence of protein-bound auxin was thus suggested, since proteolytic digestion increased the amounts of free auxin which could be subsequently extracted with ether.

Crude protein preparations were then obtained from unsteamed meals which had been extracted with ether. The usual succession of protein solvents was employed, *viz.*, water, neutral saline, alcohol and weak alkali. Tryptic digestion of these isolates likewise resulted in greater concentrations of auxin than did their respective controls. The increase of auxin yield was particularly evident with the water- and saline-soluble preparations. This further indicated the presence of auxin in protein association. In addition, the auxin seemed not to be restricted to one protein class, or, as evidenced by the meal digests, to the embryo or the endosperm protein isolates. Since procedures are in the literature for the isolation of wheat proteins in relatively pure

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[2] These materials were secured through the courtesy of Dr. C. G. Ferrari, General Mills, Inc. The germ meal was a product of 85 to 90 per cent embryo content and was not heat-treated, while the flour, a baker's patent (Washburn Special), contained approximately 13 per cent protein. Unless otherwise noted, these preparations were used for the protein isolations.

states, the study was continued with proteins obtained by such techniques.

Preparation of the proteins.—According to Osborne (1907), five unquestionably different forms of protein, differing in composition, solubility and physical characters, can be isolated from the wheat grain. These proteins are the prolamine *gliadin,* the glutelin *glutenin,* an albumin-like protein *leucosin,* a globulin, and one or more proteoses. Leucosin, globulin and proteose are obtained from the embryo of the wheat, and form all or nearly all of the protein substance of this part of the seed. Gliadin and glutenin form all or nearly all of the proteins of the endosperm, or over 80 percent of the total protein matter of the seed. The glutelin was further fractionated by Csonka and Jones (1927), who "salted out" two components at different concentrations of ammonium sulfate. These fractions they termed *α-glutelin* and *β-glutelin.*

Since Osborne's work, additional methods have been suggested for isolating some of the wheat proteins. Furthermore, it has been often noted that the technique of isolation controls to some extent the physical and chemical properties of the protein isolated. Accordingly, wherever feasible, preparations were secured of each protein used by more than one method. The isolation procedures employed duplicated closely the published methods, though sometimes several proteins were obtained from an initial dispersion. Also, wherever it seemed of value, centrifugation, supercentrifugation and pervaporation were employed. A brief outline of the isolation procedures is listed in table 1.

TABLE 1. *Outline of protein isolation procedures. Citations in parentheses refer to sources of the methods.*

Protein	Method	Per cent nitrogen
Glutenin:		
I	(Cook and Alsberg, 1931) Gliadin removed from gluten by neutral 10 per cent urea; residue dispersed in 30 per cent neutral urea, and glutenin successively precipitated by LiCl and by dilution from urea dispersions	17.3
II	(Blish and Sandstedt, 1929) Gluten dispersed in dilute acetic acid; glutenin precipitated at isoelectric point from methanol	17.4
III	(Morrow and Sandstrom, 1935) Flour dispersed in dilute alkali; glutenin precipitated at isoelectric point from methanol	17.5
Gliadin:		
I	(Dill and Alsberg, 1925) Gliadin in 10 per cent neutral urea dispersions of gluten precipitated by $(NH_4)_2SO_4$; from concentrated alcoholic solutions the gliadin was repeatedly precipitated and washed using dilute aqueous halides and absolute ethanol	17.6
II	(Blish and Sandstedt, 1926) Oven-dried gluten dispersed in dilute acetic acid; gliadin twice precipitated by absolute ethanol in presence of LiCl	17.5
α-Glutelin:		
I	(Csonka and Jones, 1927) Precipitated by 0.026 saturated $(NH_4)_2SO_4$ from alkaline dispersion of alcohol extracted gluten	17.4
β-Glutelin:		
I	(Csonka and Jones, 1927) Precipitated by 0.18 saturated $(NH_4)_2SO_4$ from filtrate after separation of α-glutelin I	16.3
II	(Csonka and Jones, 1927) Gliadin and α-glutelin precipitated from alkaline dispersion of gluten by 0.026 saturated $(NH_4)_2SO_4$; β-glutelin then precipitated by 0.18 saturated $(NH_4)_2SO_4$	16.2
Globulin:		
I	(Osborne, 1907) Albumin precipitated at 70°C. from 3 per cent NaCl dispersion of germ meal; globulin precipitated by saturated $(NH_4)_2SO_4$ and successively reprecipitated by dialysis from 10 per cent NaCl	17.9
III	(Jones and Gersdorff, 1923) Precipitated from 3 per cent NaCl dispersion of germ meal by CO_2 and acetic acid; reprecipitated from 3 per cent NaCl by 0.70 saturated $(NH_4)_2SO_4$; successively reprecipitated by dialysis from 10 per cent NaCl	18.1
IV	(Osborne, 1907) Ground whole grains, less bran, dispersed in 10 per cent NaCl; globulin precipitated by 0.80 saturated $(NH_4)_2SO_4$ and reprecipitated by dialysis from 10 per cent NaCl	18.2
Leucosin:		
II	(Osborne, 1907) Precipitated at 60°C. from supernatant fluid in first dialysis of globulin IV	16.5
III	(Osborne, 1907) Precipitated at 75°C. from filtrate obtained after precipitation of leucosin II	16.1
Proteose:		
I	(Osborne, 1907) Supernatant fluid in first dialysis of globulin I heated to 98°C.; proteose precipitated from filtrate by saturated $(NH_4)_2SO_4$; precipitate dialyzed against water and reprecipitated by dialysis against ethanol; water and ethanol dialyses repeated once	16.2

As the final steps, each protein was washed several times with 70 percent ethanol, absolute ethanol and ether. They were then dried *in vacuo* at 35 to 40°C. and ground to pass 100 mesh screens. Washes with 70 percent ethanol were omitted with the gliadin isolates. The proteins were then extracted with ether in a Soxhlet apparatus for three days, repulverized and stored over concentrated H_2SO_4. Each ether extract was assayed for growth activity by the *Avena* test using minimal amounts of agar. None displayed activity. In other words, every pro-

tein isolate used for further experiment was initially free of ether extractable auxin.

Nitrogen determinations of each protein were secured by the micro-Kjeldahl method using selenium as a catalyst. The averages of triplicate determinations are listed in table 1. These values are on a dry-weight basis but are uncorrected for ash. Though the values here listed for leucosin II and leucosin III are lower than those obtained by Osborne, they more closely approach the nitrogen content of 16.11 percent obtained by Herzner (1928) with a very pure preparation of leucosin.

Protein digestion.—Utilizing a number of the protein preparations, preliminary experiments were performed to determine some of the optimum conditions for the liberation of auxin by enzymatic digestion. The highest auxin yields were obtained under conditions similar to those giving most auxin with the leaf proteins of spinach (Wildman and Gordon, 1942). Thus, at 37°C., pH of 8.0, with varying enzyme-substrate-buffer ratios, trypsin gave higher concentrations of auxin per weight of protein substrate than crystalline trypsin or chymotrypsin (three days incubation). What is here and later referred to as "trypsin" was a commercial pancreatic preparation (Fairchild) and presumably contained a mixture of proteolytic enzymes (Tauber, 1937; Horwitt, 1945). Little difference was noted in the amounts of auxin yield when 0.1, 1.0, 5.0 and 10.0 mg. of trypsin per 10.0 mg. of protein were used, though somewhat less auxin was obtained with the lowest concentration of enzyme. Digests of both embryo and endosperm proteins, incubated with trypsin and assayed at daily intervals, attained their maximum auxin content after three to four days incubation. Unaccountably, the apparent auxin concentration decreased after five to six days incubation.

Accordingly, in the usual digestions, from 2.0 to 5.0 mg. of trypsin were used per 10.0 mg. of protein. The enzyme was dispersed in a small quantity of KH_2PO_4–NaOH buffer (pH 8.0) and slowly added to the dry protein. More buffer was then added to make a total of 10 ml. of buffer for each 10 mg. of protein. After the addition of toluol as a preservative, the digestion flask was tightly stoppered and incubated at 37°C. for 96 hours.

In the early experiments, controls of trypsin were incubated without substrate. No auxin was shown by these controls when their extracts were assayed. The enzyme controls were then discontinued on the assumption that any auxin produced in a digest would not arise from the enzyme preparation *per se*. This assumption is substantiated by the above-mentioned lack of increase in auxin content when proteins were incubated with increasing amounts of enzyme.

Auxin determinations.—Following experimental treatment, all aqueous solutions were adjusted to a pH of 4.5 (glass electrode) with HCl. The auxin was immediately removed from the aqueous phase by shaking with two 4- to 5-volume portions of purified ether. The ether was withdrawn, evaporated to a few ml. in volume, and quantitatively transferred to a small vial. A measured amount of hot 1.5 percent agar was then added, stirred, and allowed to remain at least several hours to permit diffusion of the growth substance.

Auxin assays followed the standard *Avena* procedure allowing two to two and one-half hours between decapitations of the coleoptiles and a reaction time of 90 minutes between placement of the agar blocks and photographing. Two concentrations of indoleacetic acid were assayed with every *Avena* experiment as an index of coleoptile sensitivity.

In calculating the average of the curvatures resulting from a given extract, individual values which departed from the mean by more than 2.5 times the standard deviation were discarded and the means recalculated. The concentrations of auxin are expressed in indoleacetic acid equivalents, after van Overbeek (1938). Thus, as here used, the unit "microgm. per kg." means micrograms indoleacetic acid equivalents per kg. dry weight of the material treated.

The auxin values given are averages of from two to four separate experiments. Values of the individual replicates, in turn, are based upon the means of two or three, 10- to 12- unit *Avena* rows whose curvatures were in the linear range of coleoptile reactivity. Both the quantity of substrate and the agar dilution of the hormone extract were varied to yield coleoptile curvatures within this linear range, the effective amounts of each being determined by preliminary trials.

RESULTS.—*Auxin released by tryptic digestion.*—Following the methods detailed in the preceding section, the various endosperm and embryo protein preparations were hydrolyzed by incubation with trypsin. Table 2A lists the auxin yields obtained. The data demonstrate that auxin is associated, to a greater or less extent, with each class of protein isolated from the wheat grain. Per unit of protein weight, the embryo proteins released markedly higher amounts of auxin than were secured from the proteins of the endosperm.

That the auxin release was due to enzymatic hydrolysis, and not to the slightly alkaline pH of the buffer, is indicated by the absence of auxin in the controls where the proteins were incubated without enzyme. It was shown in a previous work (Wildman and Gordon, 1942) that bacterial decomposition is probably not involved in the process.

It is also evident from table 2A that different isolation procedures for a given protein type strongly influence the quantity of auxin which can subsequently be obtained. For instance, 25 microgm. of auxin per kg. were released from the prolamine preparation, gliadin II, whereas no auxin was obtained by digestion of gliadin I. The isolation of gliadin II involved the drying and dispersal in acetic acid of the gluten, while gliadin I was secured

TABLE 2. *Auxin yields obtained by different methods of hydrolysis. A—Proteins digested by trypsin at pH 8.0. Parallel controls of each protein incubated without enzyme yielded no auxin. B—Proteins peptized in 0.01 N NaOH followed by tryptic digestion. C—Proteins autoclaved at pH 10.5. Auxin yields in microgm./kg.*

Protein	A	B		C
		NaOH pretreatment		
	Trypsin	Without trypsin	With trypsin	Alkaline autoclaving
Embryo:				
Leucosin II	300 ± 42	..	..	88
Leucosin III	320 ± 37	..	..	..
Globulin I	170 ± 9	0	..	0
Globulin III	120	..	..	..
Globulin IV	460 ± 35	0	..	0
Proteose I	410 ± 22	0	..	0
Endosperm:				
Gliadin I	0	50	53	30
Gliadin II	25 ± 2	87	89	64
Glutenin I	25 ± 3	0	13	9
Glutenin II	98 ± 5	49	125	31
Glutenin III	22 ± 3	0	31	62
α-Glutenin I	39 ± 2	..	..	..
β-Glutenin I	0	..	..	..
β-Glutenin II	0	..	..	..

from neutral urea dispersions by salting out. Approximately the same amounts of auxin were obtained from glutenins I and III, while glutenin II yielded a significantly higher quantity of auxin; I and III were prepared, respectively, by neutral and alkaline dispersion methods, whereas an acid medium was used to disperse the gluten for the isolation of II. On the other hand, globulin III, which was precipitated by acid, liberated less auxin than the other globulin isolates. The chief difference involved in preparing the albumin isolates lay in the coagulation temperatures used, which differed by 15°C. The auxin yields from leucosin II and leucosin III are virtually the same. These variations in yield from a given protein, resulting from the isolation method employed, suggested the presence of different association mechanisms between auxin and the various proteins and/or the presence of more than one auxin type.

Digestion of the α- and β-fractions of the glutelin showed that auxin was released only from the α-glutelin at the concentrations of these proteins used. Since dispersion in alkali was utilized in the preparation of the glutelin fractions, it is perhaps justified to compare their auxin yields with glutenin III. On this basis, it can be said that most, if not all, of the auxin enzymatically released from glutenin III arose from its α-fraction. The localization of auxin in a specific fraction of a protein has been previously noted with cytoplasmic proteins isolated from the leaves of spinach (Wildman and Gordon, 1942).

Relatively large amounts of auxin resulted from the digestion of globulin IV and the proteose. Globulin IV was obtained from dispersions of the whole grain and therefore can be considered free of nucleic acid, while the other globulin preparations were secured from embryo dispersions and probably contain about 15 percent nucleic acid (Osborne, 1907). Thus the higher auxin content of globulin IV, compared to the other globulin isolates, cannot be accounted for on the basis of removing a nucleic acid fraction which contains no auxin. Since Osborne's proteose preparations likewise were free of nucleic acid, it may be concluded that, insofar as indicated by enzymatic digestion of the various proteins, the globulin and proteose possess the highest auxin concentrations of the major seed proteins. These concentrations are closely approached by that of the third embryo protein, leucosin.

Auxin released by alkaline treatments.—In the preparation of the previous digestion mixtures, it was observed that some of the proteins tended to be dissolved by the buffer alone. As a whole, the embryo proteins were more easily "wet" than were the proteins of the endosperm. An effort was therefore made to provide more comparable conditions at the start of the digestion period. Each endosperm protein was peptized by prolonged stirring in 0.01 *N* NaOH to form viscous solutions containing 1 percent protein. From each solution, 10 ml. (containing 100 mg. protein) were withdrawn and neutralized by HCl. Following the procedure used with the dry proteins, trypsin and buffer were then added, the mixture incubated, and then assayed for auxin, The controls were of like amounts of protein, identically treated, but incubated without enzyme. Table 2B lists the average yields resulting from these digests.

Pretreatment with alkali resulted in the release of about 50 microgm. auxin per kg. from gliadin I, though no auxin was obtained before from this prep-

aration. Where enzymatic digestion of gliadin II, starting with the dry protein, yielded 25 microgm. auxin (table 2A), alkali prepeptization resulted in 89 microgm., more than a three-fold increase. That enzymatic digestion played no part in the auxin release from the alkali-treated gliadins is indicated by the fact that the controls equalled the enzyme digests in auxin yield. Alkaline treatment followed by digestion reduced the yield from glutenin I; however, enzymatic digests of the remaining pretreated glutenins, particularly glutenin II, contained more auxin than did digests of the same proteins which were not previously peptized in alkali. In addition, the control of glutenin II showed the presence of auxin, though in an amount less than half of that from its enzymatically hydrolyzed counterpart.

The auxin in the control of glutenin II may perhaps be explained by the hypothesis suggested by Blish and Sandstedt (1929). These workers considered that glutenin did not exist as such in the grain but was produced from a more complex body which was split by the alkali normally used as a dispersion medium. Thus, from the protein obtained by acid dispersion of the gluten (similar to the glutenin II of the present work), they could separate by subsequent alkaline treatment two proteins. One protein possessed the characteristics of glutenin secured by alkaline dispersion of the gluten; the other protein resembled gliadin. In this light one can interpret the liberation of auxin in the control of glutenin II as a function of a gliadin component, since the glutenins supposedly not in a complex association did not release auxin following alkaline peptization.

The release of auxin from some of the proteins as a result of alkaline treatment makes questionable the auxin values given for glutenin III and the α- and β-glutelin fractions in table 2A. It is probable that these values are too low, since an alkaline medium was used at some stage in the isolation of these proteins.

Increased yields of auxin from various plant tissues as a result of alkaline treatment have been noted by a number of investigators. Avery and co-workers have advocated the use of alkaline autoclaving as a means of totally converting the auxin precursor in seed materials to an extractable and active form of auxin. Therefore, to extend the alkaline treatments previously detailed, a number of the proteins isolated were subjected to autoclaving in an alkaline medium. To weighed amounts of protein were added 25 ml. of M/10 soda-borax buffer of pH 10.5. These suspensions were autoclaved at 120°C. for 15 minutes. After adjusting the solutions to a pH of 4.5, they were extracted with ether. The auxin assays of these extracts are given in table 2C.

Immediately evident is the fact that alkaline treatment at an elevated temperature caused auxin to be released from both leucosin and the endosperm proteins but not from globulin and proteose. When 100 mg. of ground wheat grains plus 80 mg. of proteose were autoclaved under the same conditions, no increase of auxin was noted compared to the grain autoclaved alone. Both procedures yielded 800 microgm. auxin per kg., showing that the presence of other substances in the seed would not cause the proteose to release auxin by this alkaline treatment.

Autoclaving under alkaline conditions, as compared with the alkaline prepeptization treatment, reduced the auxin yields from both gliadins and from glutenin II. With glutenin I there was no significant change, while glutenin III was the only protein which showed an increase of auxin yield. Although auxin was obtained by autoclaving leucosin, the yield was much less than that which arose from enzymatic digestion alone. For the endosperm proteins as a group, one may conclude that the alkaline autoclaving method was a less effective mechanism for auxin release than the alkaline pretreatment procedure, or that destruction of the auxin produced occurred.

To interpret the varying yields of auxin, two possible explanations were considered: (1) One type of auxin is common to all the proteins; it is liberated from some of the proteins by enzymes and from the others by alkali. (2) At least two types of auxin are present; one is specifically released by enzymes (or is destroyed by alkali), while the other is liberated both enzymatically and by alkali.

The occurrence of more than one type of auxin seemed to be a more tenable hypothesis on the basis of previous work. Gordon and Wildman (1943) observed that alkaline hydrolysis of the cytoplasmic proteins of spinach released an auxin with a diffusion rate similar to indoleacetic acid. The auxin secured from the spinach proteins by enzymatic action possessed a markedly different rate of diffusion. Furthermore, Avery, Berger and Shalucha (1942) concluded from their data that two auxins were present in the wheat grain, one of which showed the properties of alkali-stable indoleacetic acid, the other being alkali-labile. Haagen-Smit *et al.* (1941, 1942) isolated both indoleacetic acid and pseudoauxin *a* from corn; Berger and Avery (1944a) later confirmed the presence of the former auxin in extracts of corn which had been hydrolyzed by alkali.

Auxin sensitivities to acid and alkali.—In view of the above considerations, it seemed pertinent to determine whether one or more than one type of growth substance was released from the various wheat proteins, and if more than one type occurred, whether the method used to obtain auxin had any effect on the nature of the hormone secured, both from the proteins and from the grain.

To indicate the pH sensitivity of the auxin in a given extract, the procedure used by van Overbeek and Bonner (1938) was followed. However, in the present tests, the auxin extracts were refluxed for 15 minutes in 5 ml. of *N* HCl, *N* NaOH, and distilled water. Reflux experiments were performed in replicate on (a) the auxin released by tryptic digestion of various proteins; (b) "free" auxin obtained by a short ether extraction of ground wheat grains;

TABLE 3. *Sensitivity to acid and alkali of the auxins obtained by (a) digestion of untreated proteins, (b) ether extraction of the grain, (c) digestion of proteins previously peptized in alkali, (d) autoclaving the grain at pH of 10.5.*

Auxin source	Microgm./kg. auxin			
	Not refluxed	H_2O reflux	HCl reflux	NaOH reflux
a. Gliadin II	21	18	7	0
Glutenin II	91	93	69	0
Leucosin II	310	160	140	0
Globulin I	140	99	0	130
Globulin IV	340	170	0	200
Proteose I	350	280	0	310
b. Grain (ether extract)	32	26	19	5
c. Gliadin II	87	62	0	60
Glutenin II	100	87	0	76
Leucosin II	180	120	10	100
d. Grain (autoclaved)	730	740	0	770

(c) the auxin secured by digestion of gliadin, glutenin and leucosin which had previously been peptized in alkali. The resultant data are given in table 3.

Section *a* of table 3 is striking in its indication that two types of auxin were released by enzymatic digestion of the proteins not pretreated. From the endosperm proteins and from leucosin was liberated auxin which was completely inactivated by reflux in alkali. On the other hand, the auxin released from the globulin and proteose was wholly inactivated by the acid reflux. Some acid-labile auxin also may have been produced from the former group of proteins, in view of the partial inactivation which occurred when their auxin was refluxed in acid.

That there are likewise two auxin types in the free auxin of the grain is indicated by section *b* of table 3. Part of the auxin extracted by ether from the grain is sensitive to acid, while the greater part is inactivated by alkali. These results substantiate the afore-mentioned conclusion of Avery *et al.* (1942) which was based on the different auxin yields obtained by hydrolysis of the wheat grain with varying strengths of alkali. They estimated from their ratios of yields that 70 per cent or more of the "total" auxin of the seed is a compound other than indoleacetic acid, i.e., is alkali-labile. According to the present data, nearly the same ratio holds for the free auxin.

Avery *et al.* (1942) also concluded from their different yields "that two auxins differing markedly in their alkali-stability are formed upon mild hydrolysis [of the wheat grain] with alkali." Examining the data in section *c*, table 3, it is evident that a treatment involving exposure to alkali did not release two kinds of auxin from leucosin and the gluten proteins. Digests of these proteins which had been previously peptized in alkali yielded auxin which was heat-stable in alkali but labile in acid. However, the present data indicate that two auxins of differing pH stabilities are associated with gliadin, glutenin and leucosin; tryptic digestion liberates predominantly the auxin inactivated by alkali (section *a*, table 3), while the treatment involving alkaline peptization releases chiefly an alkali-stable auxin.

Considering the above results, it was decided to investigate further the acid-alkali sensitivity of the auxin produced by alkaline autoclaving of the grain, actually subjecting the auxin extracted to extremes of pH. Accordingly, 100 mg. of ground grain were suspended in 25 ml. of soda-borax buffer, pH 10.5, and autoclaved 15 minutes at 120°C. After cooling, the suspension was adjusted to a pH of 4.5 and extracted with ether. The ether extract was treated as described before to determine the auxin's stability in acid and alkali. As shown in section *d*, table 3, the results of this experiment are at variance with the conclusion of Avery, Berger and Shalucha (1942) that two auxin types are formed upon alkaline hydrolysis of the wheat grain. The present data indicate that all of the auxin found upon autoclaving the grain at a pH of 10.5 is heat-stable in alkali and inactivated by acid, a property characteristic of indoleacetic acid.

The auxin yields obtained by Avery *et al.* upon autoclaving the wheat grain in *N* NaOH were from 15 to 69 percent of the maximum yields which resulted from autoclaving in buffer at a pH of 10.5. Since indoleacetic acid is stable in alkali, they inferred that destruction of an auxin had occurred at the higher alkalinity, and that this alkali-labile auxin comprised the greater portion of the growth substances present in the hydrolysates obtained by the less alkaline treatment. In the light of the present experiments, in which it is shown that the auxin obtained from the grain by alkaline autoclaving at pH 10.5 is heat-stable in alkali, it seems more reasonable to assume that the large amounts of auxin released by alkaline treatments are chiefly of the indoleacetic type, and that the higher the alkalinity of the autoclaving medium, the less effective is the conversion of the parent substance into the auxin

produced, although a concurrent reaction may be destruction of alkali-labile auxin.

Electrodialysis.—In a previous paper (Wildman and Gordon, 1942), the possibility was considered that the association of auxin with the leaf proteins of spinach was an adsorption phenomenon. However, though the auxin liberated by digestion of these proteins was soluble in ether and ether-alcohol, prolonged extraction of the proteins by the same solvents failed to elute any auxin. While isolating the proteins used in this study, an attempt was made to remove the halide from a globulin dispersion by electrodialysis. When the electrodialyzed protein was subsequently incubated with trypsin, no auxin could be detected in the resulting digest. This suggested the existence of the auxin as an adsorbed electrolyte on the protein molecule, capable of being removed by the use of an impressed electrical potential.

Consequently, the procedure used to obtain globulin IV was repeated. Precipitated globulin spheroids resulting from the first dialysis were dissolved in 10 percent NaCl and the solution filtered clear. The filtrate was divided, one portion being dialyzed as usual against chilled tap and distilled water. The remaining portion was electrodialyzed in an apparatus whose cell was constructed according to Bartell (1936). Electrodialysis, with the current intensity not exceeding 400 milliamperes, was continued till the solution showed negligible conductivity. By governing the rate of water flow against the collodion membrane, the temperature of the protein solution was kept at all times below 30°C. Dialyzed and electrodialyzed preparations of proteose were likewise obtained, the final precipitates being secured by dialysis against ethanol. Each protein was dried *in vacuo,* ground and extracted with ether for three days. No auxin was found in any of the ether extracts.

In order to obtain an electrodialyzed endosperm protein, a quantity of glutenin II was peptized as far as possible in *N* KI and the solution filtered clear. One part of the filtrate was dialyzed against chilled tap and distilled water. The remaining part was electrodialyzed.

Following the usual procedure, the various preparations were incubated with trypsin and the digests assayed for their auxin content. The glutenin isolates were also autoclaved at pH 10.5, 120°C., for 15 minutes.

As shown in table 4, no auxin was detected in the enzymatic digests of the globulin and proteose which were electrodialyzed. Thus it seems probable that the auxin is directly or indirectly associated with these proteins by adsorption, or more specifically, by some linkage sufficiently stable to resist elution by ether but not sufficiently stable to withstand electrodialysis. That a decreased digestibility of the electrodialyzed proteins was not the cause of lack of auxin release was indicated by amino acid assays of dialyzed and electrodialyzed protein incubates. Van Slyke analyses of globulin (d) and globulin (ed) digests, after 100 hours incubation, showed amino acid contents that differed by about 10 per cent. Likewise, the digests of glutenin (d) and glutenin (ed) differed by about 14 percent. More conclusive evidence as to the existence of an adsorption complex would have been the recovery of auxin from the electrodialysates, a task which was not attempted.

Electrodialysis of glutenin caused a two-thirds reduction in auxin yield when this protein was digested by trypsin. However, no auxin was found when the electrodialyzed glutenin was autoclaved in alkaline buffer. It was previously shown that the auxin enzymatically released from globulin and proteose was alkali-stable, as was also the auxin secured from glutenin by a treatment incorporating exposure to alkali. On the other hand, the auxin liberated from glutenin by tryptic digestion was shown to be predominantly alkali-labile. Hence, the auxin yield in the tryptic digest of the electrodialyzed glutenin may indicate a more stable binding of the alkali-labile auxin to this protein. In this connection the fact may be mentioned that glutenin is extremely insoluble in water (Osborne, 1907). Upon electrodialysis, it flocculated with great rapidity—a phenomenon which would undoubtedly decrease the extent of hydrogen replacement of adsorbates, particularly those more "tightly" bound to the protein. The fact that a decreased auxin yield did occur in the tryptic digest of glutenin (ed) reduces the likelihood that the alkali-labile auxin is an internal and integral constituent of the glutenin molecule.

TABLE 4. *Auxin yields from proteins after being dialyzed (d) and electrodialyzed (ed).*

Protein	Auxin yield microgm./kg.
Tryptic digestion:	
Globulin (d)	410
Globulin (ed)	0
Proteose (d)	450
Proteose (ed)	0
Glutenin (d)	83
Glutenin (ed)	28
Autoclaved at pH 10.5:	
Glutenin (d)	29
Glutenin (ed)	0

The rate of auxin release and protein hydrolysis.—The electrodialysis experiments indicated that auxin occurs adsorbed to the proteins. Still, if auxin were a recurrent unit in the protein chain, one would expect to find during enzymatic hydrolysis of the protein that the concentration of auxin in the hydrolysate would increase at a rate roughly parallel to the increase of amino acids. When protein hydrolysis becomes markedly slowed, as would be indexed by the concentration of free amino acids, the auxin concentration should likewise become uniform.

The concentrations of amino acids and auxin were therefore periodically determined in protein digests. The proteins used were glutenin II, most of whose auxin liberated by enzymes is alkali-labile, and globulin IV, which was shown to release alkali-stable auxin. Series of digests were incubated in which the concentration of protein was 2.0 mg. per ml. of buffer and the amount of enzyme per 5 ml. of buffer was 1.0 mg. As before, phosphate buffer of pH 8.0 was used, and the suspensions were incubated at a temperature of 37°C. Controls of trypsin were run simultaneously to correct for enzyme autolysis. At the time of sampling, the entire liquid of the digestion flask was filtered without washing. From the filtrate a suitable aliquot was taken for amino-nitrogen assay by the method of Van Slyke (Morrow and Sandstrom, 1935). After this determination, the filter was washed; the combined washings and original filtrate were adjusted to pH 4.5 and immediately shaken with ether. The ether solution was then treated according to the usual procedure for auxin assay. Concentrations of amino nitrogen and auxin after various intervals of digestion are presented in figure 1.

Undoubtedly auxin was released earlier than the time indicated by the beginning of the globulin-auxin curve in figure 1. However, the aliquots taken from the hydrolysate for Van Slyke analysis probably left too small an amount of auxin in the remainder for the *Avena* coleoptiles to react.

It will be recalled that no auxin was obtained from untreated proteins incubated without enzyme. Thus the dependence of auxin release upon digestion of the proteins in this experiment is obvious. Nevertheless, the concentration of amino-N in the glutenin digests became virtually constant at about 24 hours, whereas the auxin concentrations in these digests attained maximal values at about 110 hours, a difference of roughly three and one-half days. To a lesser extent, the same phenomenon was characteristic of the globulin protein. Leveling of the amino-N curve for globulin occurred at about 35 hours, while the auxin concentrations can be estimated as reaching peak values in the region of 60 to 90 hours, a difference of at least one day.

From these results it may be concluded that active auxin and amino acids are not released from globulin and glutenin at similar rates. Yet, if auxin is associated with the proteins by surface forces and released upon protein hydrolysis, one would also expect auxin concentrations to attain their maximal values close to, or even before, the slowing of peptide bond hydrolysis. The fact that the auxin maxima are considerably delayed suggests that either an inactive form of auxin is released, which is subsequently converted to the active state (resembling, in this respect, the inactive corn-endosperm "precursor" of Avery and co-workers which readily becomes active under mildly alkaline conditions), or that the auxin is part of a fraction, enzymatically more slowly hydrolyzed, which is common to all of the protein classes isolated.

Discussion.—In a recent review, van Overbeek (1944) classified the auxin precursors of plants into two groups: inactive, nonprotein *endosperm precursor*, and inactive, protein *leaf precursor*. It has been shown in the present investigation that inactive auxin-protein complexes occur in the grain of wheat. There appears no reason why these complexes should not be considered as possible precursors of the free auxin in the grain, in view of the fact that the free auxin contains two types of growth sub-

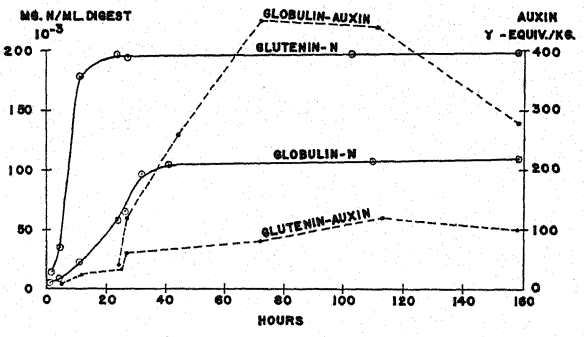


Fig. 1. Amino-N and auxin concentrations in protein hydrolysates after various incubation periods. Amino-N corrected for blank and enzyme autolysis.

stance, both of which were shown to be associated with the proteins isolated. In addition, auxin can be obtained from some of the proteins of the seed, as well as from leaf proteins (Gordon and Wildman, 1943), by alkaline hydrolysis. Thus, as van Overbeek anticipated in discussing the above categories, the two classes of precursor cannot be definitely characterized on the basis of being protein or nonprotein, or upon the different ways they can yield active auxin.

The presence of auxin-proteins in plants could likewise be interpreted as a result of auxin function. It has been suggested that auxin acts as an exchange adsorbate of protein-adsorbed enzymes (Eyster, 1943), as a coenzyme (Skoog, Schneider and Malan, 1942) or enzyme activator (Berger and Avery, 1943), and as a protein dissociant (Northen, 1942). Auxin-protein complexes might arise in each of these reactions. Since there is still insufficient direct evidence as to how auxins function in the numerous phenomena which they apparently condition, it seems more acceptable at this time to regard auxin-proteins as likely precursors.

Such a view is also tenable from a physiological standpoint. The proteolysis accompanying germination, if one may reason by analogy from the *in vitro* experiments, would concurrently liberate auxin. Relatively high concentrations of auxin in the embryo proteins suggest that a source of auxin is immediately available to the embryo at resumption of its growth.

It should be emphasized that the proposal of auxin "parent" substances or fractions adsorbed to the proteins is only tendered as one explanation of

the experimental data. We may say with surety that auxin is associated with the proteins isolated, and that the indications are that it is adsorbed. Whether the auxin is present in the complex as auxin, *i.e.*, without significant structural change, or as some modification which is converted into auxin after being released requires further elaboration.

The conclusion of Avery, Berger and Shalucha (1942) that two auxin precursors were present in the wheat grain was formulated as follows: (1) Since different auxin yields at various pH levels indicated the presence of two auxins, it was logical to assume that they were derived from different precursors. (2) The auxin content of a water extract of wheat was determined. The same extract, after alkaline autoclaving, showed an auxin increase of over five-fold, demonstrating the presence of a water-soluble precursor. However, since suspensions of the grain autoclaved at an alkaline pH gave over three times the auxin yield of the autoclaved water extract, they surmised that not all of the wheat precursor was readily water-soluble.

Considering the auxin-proteins as precursors corroborates the above conclusion as to the existence of two precursor types from a qualitative view. It was shown that both alkali-labile and alkali-stable auxins were associated with gliadin, glutenin and leucosin, and that alkali-stable auxin could be obtained from the globulin and proteose. The water extract of wheat that Avery *et al.* used undoubtedly contained leucosin, a protein which will yield auxin upon autoclaving in mild alkali. When they autoclaved a suspension of wheat, a still greater yield of auxin would be expected, since both glutenin and gliadin, the chief proteins of the grain, likewise yield auxin upon alkaline autoclaving (cf. table 2C). On a quantitative basis, however, the auxin yields obtained from the whole grain by these workers and others, and enzymatically or by alkaline treatment in the present work, cannot be accounted for by the yields from the isolated proteins.

Furthermore, Avery *et al.* (1942) found that the auxin yields obtained by alkaline-autoclaving six different varieties of wheat were unrelated to the protein contents of the wheats. Since gliadin, glutenin and leucosin, which liberate auxin by alkaline treatment, form about 85 to 90 percent of the protein matter of the grain (Osborne, 1907), a limited proportionality relationship would be expected. The following alternative conclusions may therefore be drawn: (1) The yields secured from the isolated proteins are much lower than the auxin concentrations of the same proteins in the grain. As an adsorption phenomenon, the amounts of auxin associated with these proteins may vary with the variety of grain sufficiently to offset any correlation between total protein and auxin yield. (2) There are present in the grain other sources of auxin from which auxin can be released by proteolytic enzymes or by alkaline hydrolysis. The concentrations of such sources need not be related to the grains' total protein content.

The above mentioned lack of relationship also raises the question as to whether the auxin found associated with the proteins is simply auxin adsorbed during the process of protein isolation. Weighing against this is the evidence that auxin is in protein association in a number of types of plant tissues. Increased auxin yields upon proteolytic digestion were noted with seeds in the present work and by Haagen-Smit *et al.* (1942); with other plant tissues by Skoog and Thimann (1940), Thimann *et al.* (1942), Judkins (1945), and with isolated intact leaf chloroplasts by Wildman and Gordon (1942).

In regard to the auxin release from some of the proteins by alkaline treatment, it is pertinent to mention tryptophane as an auxin precursor. According to Schmidt (1938), the glutenin of wheat has a tryptophane content of 1.7 percent. Auxin was liberated upon alkaline autoclaving of a glutenin isolate which was not electrodialyzed. The same treatment of electrodialyzed glutenin yielded no auxin. In addition, no auxin was detected when globulin and proteose were subjected to alkaline treatments. These results indicate that the tryptophane native to these proteins did not function as the origin of auxin upon alkaline treatment, contrary to the idea expressed by Gordon and Wildman (1943). These workers' criticism of the alkaline extraction method solely on a basis of tryptophane conversion to auxin has been also greatly weakened by various data which have been since presented (Avery and Berger, 1943; Berger and Avery, 1944b; Avery, Berger, and White, 1945), though tryptophane is undoubtedly converted to some extent under alkaline conditions (Gordon and Wildman, 1943; Berger and Avery, 1944b).

Nevertheless, if the aim of an extraction method is to obtain "total" auxin, the use of alkaline hydrolysis is still open to question. It was shown in the present work that two auxin types are associated with some of the proteins, whereas only one auxin appeared in the digests of these same proteins as a result of alkaline treatment. Auxin was obtained by enzymatic digestion of globulin and proteose but not by the use of alkaline media, and though two auxins are present in the grain in the free form, only one auxin appeared in the alkaline hydrolysate of the whole grain. Moreover, the auxin released by alkali from leaf proteins of spinach possessed a diffusion rate similar to indoleacetic acid, whereas an auxin of dissimilar diffusion characteristics was obtained by enzymatic digestion (Gordon and Wildman, 1943). These results indicate that the method of alkaline hydrolysis does not yield a valid qualitative estimate of the total auxin concentration, even though relatively great amounts of auxin may be secured.

SUMMARY

Auxin was found to be associated with each of the five major proteins isolated from the wheat grain.

Considerably more auxin was obtained from the albumin, globulin and proteose of the embryo than from the prolamine and glutelin of the endosperm by tryptic digestion. Auxin was secured only from the α-fraction when the α- and β-components of the glutelin were enzymatically hydrolyzed (table 2A).

Auxin could be obtained from the globulin and proteose by enzymatic digestion but not by alkaline treatment, while with the prolamine, glutelin and albumin either method yielded auxin (tables 2A, 2B, 2C).

Two types of auxin were found: (1) acid-stable, alkali-labile; (2) acid-labile, alkali-stable (indoleacetic acid type). Both types were present in the free auxin of the grain. By tryptic digestion, type (2) was obtained from the globulin and proteose, while type (1), and possibly type (2), were secured from the prolamine, glutelin and albumin. Only type (2) was obtained by alkaline treatment of the latter proteins and by alkaline treatment of the whole grain (table 3).

Electrodialysis experiments (table 4) indicated that auxin is associated with the proteins as an adsorbed or unstably bound electrolyte. Though digestion of the various proteins was necessary to obtain specific types of auxin, there was no correspondence between the rates of protein hydrolysis and auxin formation (fig. 1).

The implications of the above results to methods of auxin extraction and to some concepts of auxin origin are discussed.

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RELATION OF ANAEROBIC TO AEROBIC RESPIRATION IN SOME STORAGE ORGANS WITH SPECIAL REFERENCE TO THE PASTEUR EFFECT IN HIGHER PLANTS [1]

C. O. Appleman and Russell G. Brown

PASTEUR DEMONSTRATED the inhibiting effect of oxygen on yeast fermentation and claimed that inhibition of fermentation by oxygen is a phenomenon present in all living cells able to ferment and oxidize carbohydrates. The Pasteur effect has been variously defined by modern workers. Dixon (1937) defined it as the action of oxygen in diminishing carbohydrate destruction and in suppressing or decreasing the accumulation of the products of anaerobic metabolism. Marsh and Goddard (1939) state that an increased rate of sugar decomposition in transferring tissues from air to nitrogen is known as the Pasteur effect. Burk (1939) reverts to Pasteur's original conception and defines the Pasteur effect as oxygen inhibition of the fermentative processes.

The Pasteur effect was the basis for Meyerhof's theory (1925) of oxidative resynthesis in carbohydrate metabolism, and the Meyerhof cycle has been widely accepted as the explanation of the Pasteur effect. Meyerhof's theory was elaborated somewhat by Blackman (1928) as oxidative anabolism, and incorporated by him into a general scheme of the respiratory process in higher plants. Blackman's oxidative anabolism is not essentially different from Meyerhof's oxidative resynthesis.

Various steps in carbohydrate metabolism have been proposed as the place where the paths of fermentation and respiration diverge. According to Blackman's scheme, the divergence occurs after irreversible glycolysis of the sugar molecule, the glycolytic products being common to both fermentation and respiration. In the presence of oxygen, part of these products are worked back into the system by oxidative anabolism. Blackman's oxidative anabolism theory was based largely on respiration data with apples (Parija, 1928). These plant organs exhibit a pronounced Pasteur effect but so far only a few other cases of a clearly demonstrated Pasteur effect in higher plants have been reported.

The chief purpose of the present study was to test the Pasteur effect in a number of different storage organs, chiefly vegetables, under a wide variety of circumstances. Secondary consideration was given to the validity of the ratio of anaerobic to aerobic respiration as supporting experimental evidence for the unitary theory of anaerobic and aerobic respiration in higher plants.

In this paper, the term anaerobic respiration refers to the liberation of carbon dioxide from tissues in nitrogen. There is no inference regarding the metabolic source of the carbon dioxide except that molecular oxygen is not involved in any of the energy-releasing processes. Aerobic respiration is applied to the liberation of carbon dioxide from tissues in air. The metabolic source of the carbon dioxide includes reactions involving oxygen directly or indirectly as well as any reactions liberating carbon dioxide in which oxygen is not involved as one of the reactants.

MATERIALS AND METHODS.—Anaerobic respiration was measured concurrently with the normal aerobic respiration in the following plant organs under the conditions stated: potatoes (*Solanum tuberosum* L.), turnips (*Brassica rapa* L.), parsnips (*Pastinaca sativa* L.), and onions (*Allium cepa* L.), after short exposures to low temperature to give the "change of temperature effect"; potatoes after long periods of storage under different temperature and moisture conditions; potatoes, carrots (*Daucus carota* L.) and parsnips at a high and low respiration temperature; potatoes before and after treatment with ethyl bromide gas and after wounding; green sweet corn at different stages of ripening and during storage at high and low temperatures; mature sweet corn and Reid's yellow dent corn (*Zea mays* L.) containing different percentages of imbibed water; tomatoes (*Lycopersicon esculentum* L.) at different stages of ripeness and at a high and low respiration temperature. An opportunity was thus afforded to compare anaerobic respiration with aerobic respiration in the same organ under different circumstances and in different organs with wide variation in respiratory intensity.

The time rate of respiration was determined by measuring the amount of carbon dioxide liberated from a unit of plant tissue in unit time. During the measurement of anaerobic respiration, nitrogen free of oxygen and carbon dioxide was passed over the respiring material. Traces of oxygen present in the tank nitrogen were removed by passing the gas over copper, heated to about 400°C. in an electric combustion furnace. To insure greater surface area of copper for combining with oxygen, a quantity of granular cupric oxide was placed in a long tube of copper screen cloth, which in turn was placed in a glass combustion tube. Since the copper was oxidized with use, its reduction with hydrogen was repeated as often as necessary. After passing over the copper, the nitrogen was drawn through a tube of soda lime, a bottle of alkaline pyrogallol, and another of baryta water to insure removal of both carbon dioxide and traces of oxygen.

For accurate control of the gas flow through the respiration chambers, a flow meter was used in each air or nitrogen line. The flow of gas was two to five liters per hour, depending on the intensity of the

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respiration of the experimental material. The gas flow was kept as constant as possible when a series of experiments were to be compared.

The period during which either CO_2-free air or nitrogen was drawn over the samples previous to the collection of the expired CO_2 varied from 18 to 24 hours. The air surrounding the anaerobic samples had to be replaced completely by nitrogen, and the oxygen dissolved in the plant sap allowed to diffuse out before true anaerobic conditions actually obtained within the tissue. During this preliminary period, the nitrogen was drawn through the respiration chambers at the same rate as that during the respiration measurements. The method employed to measure the amount of expired CO_2 was similar to that described by Gore (1911).

The air or nitrogen after leaving the respiratory chambers was bubbled through 100 cc. of approximately normal sodium hydroxide. At the end of every 24-hour period the collected CO_2 was measured. Barium chloride was added, in excess, to the sodium hydroxide solution. In the presence of the precipitated barium carbonate, a double titration with phenolphthalein and methyl orange as indicators was made with normal hydrochloric acid. The absorption of the CO_2 in the soda solution was accomplished in a Reiset absorption apparatus. (Hempel and Dennis, 1911). Reiset tubes were fixed vertically in a side-arm flask by a gasket made from a rubber stopper. The Reiset tubes were similar to those used by Reiset except that three discs of platinum gauze of about 35 mesh per cm. were fused in the glass tubes, one on the bottom, the other two at 12.5 and 25 cm. respectively from the bottom. The tubes were 46 cm. high and 2.1 cm. inside diameter. The method and apparatus used in this laboratory have been described in detail by Kimbrough (1925).

In all of the experiments except with Dent corn, the results were calculated to a unit of wet weight. The stated hourly CO_2 output per unit weight of material is the hourly average for the 24-hour period. The successive periods 1, 2, 3, etc., in the tables mean that the same material is respiring for successive 24-hour periods.

Boswell and Whiting (1940) claim that reduced basicity of potato tissue in nitrogen liberates bound carbon dioxide, and this accounts for the output of carbon dioxide in nitrogen in excess of the air value during the early stages of anaerobic respiration. They measured carbon dioxide output during the hours immediately after transference of the tubers from air to nitrogen. By the end of seven hours in nitrogen the carbon dioxide output was even below that in air. Any chemically bound carbon dioxide would, therefore, have been liberated during the first seven hours in nitrogen. In the experiments in the present paper, the bulky tissues were in nitrogen from 18 to 24 hours before the measurements of carbon dioxide output were begun. It was assumed, therefore, that the carbon dioxide output in nitrogen after this preliminary period in nitrogen was entirely from cell respiration.

The Pasteur effect in animals and lower plants is usually demonstrated by the difference in the amounts of fermentation products formed in nitrogen and in air. Aerobic fermentation under normal aerobic conditions has not been demonstrated in higher plants except in some seeds. Fermentation products produced in nitrogen have not been identified or even detected in some of the organs included in this study.

The determination of the increased rate of sugar decomposition in transferring the tissues from air to nitrogen is not feasible in organs that have a large reserve of polysaccarides easily converted into sugar. The sugar content in parsnips, potatoes and sweet corn actually increased during the short period in nitrogen due to the hydrolysis of starch. In the present study, the Pasteur effect is demonstrated by the ratio CO_2 output in nitrogen/CO_2 output in air. This ratio is expressed as I/N.

If the sole source of CO_2 in nitrogen is alcoholic fermentation of sugar by way of pyruvic acid and acetaldehyde then a I/N ratio of .33 would indicate that the same amount of sugar is consumed in unit time in normal aerobic respiration and in anaerobic respiration and the Pasteur mechanism would not be operating. It is logical to conclude that more sugar would be consumed per unit of CO_2 produced in nitrogen than in air, even though alcohol or other cleavage products cannot be detected. The Pasteur effect would then be definitely demonstrated when the I/N ratios are greater than unity. If alcoholic fermentation is the sole source of CO_2 in nitrogen, an I/N ratio less than 1 but somewhat greater than .33 would indicate the Pasteur effect, but with our present imperfect knowledge of the mechanism of anaerobic respiration in the plant organs included in the present study, such ratios are difficult to interpret in relation to the Pasteur effect.

Potatoes (*Solanum tuberosum*).—Maryland certified Irish cobbler and McCormick potatoes were used for most of the potato experiments. The Russet Burbank variety furnished the material for a few experiments. The Maryland certified Irish Cobbler seed potatoes were dug November 18, the tops having died naturally before digging. Until further treatment, the tubers were placed in ventilated crates which were stored in the laboratory and protected from the light. The Maryland certified McCormick potatoes were dug November 6 and stored in a cool cellar until the date indicated for the beginning of each experiment. The Russet Burbank potatoes were purchased on the local market. Unless otherwise stated, the experimental lots always consisted of eight tubers each and the lots ranged in weight from about 900 to 1,000 g.

Previous cold storage.—Previous work by Appleman and Smith (1937) seemed to prove conclusively that the initial high CO_2 output from potatoes when

TABLE 1. *Respiration at 22°C. in Russet Burbank potatoes before and after cold storage.*

Previous treatment	Respiration periods of 24 hours	CO_2 per kilogram per hour		
		Aerobic	Anaerobic	I/N
		mgm.	mgm.	
As purchased on the market	1	9.9	8.9	.90
	2	9.4	6.5	.70
	3	8.8	7.1	.81
Stored at 2.2°C. for 25 days beginning December 12	1	33.6	12.6	.37
	2	35.8	12.2	.34
	3	29.9	10.2	.34
	4	23.8	8.6	.36
Stored at 2.2–2.8°C. for 35 days beginning December 12	1	26.7	12.3	.46
	2	24.5	11.6	.47
	3	21.6	11.9	.55
	4	15.9	11.3	.71

they are transferred from a low to a higher constant temperature is due to vital activity and not simply to the lower solubility of CO_2 at the higher temperature. Potatoes were stored for a time at a low temperature and then the time rate of anaerobic as well as aerobic respiration in the potatoes was determined at a constant temperature of 22°C. The variety, previous storage treatments, and the results are presented in tables 1, 2 and 3.

When the temperature of the cold storage potatoes was raised quickly to 22°C., the initial high CO_2 output occurred in both air and nitrogen, but the "change of temperature effect" was greater on the aerobic than on the anaerobic respiration. In the McCormick variety the I/N ratios before cold storage were greater than unity and the difference in the effect of previous cold storage on the aerobic and anaerobic respiration was less than in the other two varieties. The I/N values actually increased during the period of the respiration tests because of the rapid falling with time of the aerobic respiration from the initial high value.

Long periods of moist and dry storage.—McCormick potatoes were stored at room temperature in air and in moist sawdust for 62 and 90 days. The air-stored lots were placed in wire test tube baskets and protected from light. The lots stored in moist sawdust were placed in large flower pots. Water was added occasionally to keep the sawdust moist. At the end of the first storage period, the tubers in moist sawdust were beginning to sprout; at the end of the second storage period, the tubers in the air bore sprouts about 1 cm. long, while those in moist sawdust bore sprouts from 3 to 8 cm. long. The sprouts were not removed before the respiration test. The results are presented in table 4.

The extensive sprout growth on the tubers stored in sawdust did not increase the anaerobic respiration in these tubers. The aerobic respiration was slightly greater in the sprouting tubers; therefore, the I/N

TABLE 2. *Respiration in Irish Cobbler potatoes at 22°C. before and after cold storage.*

Storage	Respiration periods of 24 hours	CO_2 per kilogram per hour		
		Aerobic	Anaerobic	I/N
		mgm.	mgm.	
Immediately after harvest on November 18	1	12.1	11.1	.92
	2	11.5	10.1	.88
	3	10.5	9.4	.90
	4	9.6	9.5	.98
	5	8.9	7.8	.87
Room temperature (mean 18°C.) for 54 days	1	5.0	6.0	1.20
	2	5.0	3.5	.71
	3	4.8	3.1	.64
	4	4.8	3.2	.65
	5	5.0	3.3	.66
Room temperature (mean 18°C.) for 47 days, then at 2.5°C. for 56 days	1	28.7	12.6	.44
	2	29.5	12.1	.41
	3	24.8	11.1	.45
	4	20.2	11.6	.58
	5	15.0	11.2	.71

TABLE 3. *Respiration in McCormick potatoes at 22°C. after varying periods of storage at warm and cold temperatures.*

Storage treatments		Respiration periods of 24 hours	CO_2 per kilogram per hour		
Temperature	Storage period		Aerobic	Anaerobic	I/N
			mgm.	mgm.	
22 ± .5°C.	21 days—Nov. 20 to Dec. 11	1	6.4	8.1	1.26
		2	6.3	7.5	1.18
		3	6.5	9.2	1.42
		4	6.1	7.8	1.28
	49 days—Nov. 20 to Jan. 8	1	4.5	6.1	1.35
		2	4.9	5.6	1.14
		3	5.0	5.2	1.03
		4	4.8	7.1	1.47
1.5 ± .5°C.	21 days—Nov. 20 to Dec. 11	1	29.3	23.6	.80
		2	28.5	23.1	.81
		3	23.3	18.9	.81
		4	18.1	17.3	.96
	49 days—Nov. 20 to Jan. 8	1	32.0	23.1	.72
		2	37.4	24.0	.64
		3	31.0	21.3	.69
		4	24.7	20.4	.83
	76 days—Nov. 20 to Feb. 5	1	30.1	21.1	.76
		2	35.7	22.6	.63
		3	33.7	23.0	.68
		4	27.0	20.6	.76

ratios decreased in value with long storage in moist sawdust.

High and low respiration temperature.—The purpose of these experiments was to compare the I/N values for potatoes respiring at two widely different temperatures. The respiration temperatures and the results are presented in tables 5, 6 and 7.

The average of the I/N values for the first three days of the respiration period was practically the same for Russet Burbank potatoes respiring either at 22°C. or at 2.2°C. For the McCormick potatoes the average of these values for the same period was slightly greater at 2°C. than at 22°C. The results also show that both aerobic and anaerobic respiration in potatoes at 22°C. is high immediately after harvest.

The aerobic respiration of McCormick potatoes at low temperature decreased gradually with long storage at the same low temperature, but the anaerobic respiration for the first three days of the tests remained fairly constant throughout the storage period. The increase in the I/N ratios with each test period is due to the decrease in aerobic respiration.

Ethyl bromide treatment.—Appleman (1916) found that the normal aerobic respiration of potatoes was greatly increased for a time after the tubers had been treated with ethyl bromide gas. The following experiment was planned to test the effect of

TABLE 4. *Respiration of McCormick potatoes at 22°C. after storage in air, and in moist sawdust at room temperature for 62 days.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	After storage in air			After storage in moist sawdust		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	4.6	5.8	1.26	8.1	8.0	.99
2	5.2	5.4	1.03	7.6	5.8	.77
3	4.4	5.5	1.24	6.1	5.5	.90
4	4.9	7.5	1.55	6.7	6.5	.97
			Stored 90 days			
1	5.6	6.7	1.20	9.1	8.8	.96
2	5.7	5.4	.93	8.9	6.8	.76
3	5.7	6.3	1.11	8.5	5.3	.63
4	5.4	6.6	1.21	8.0	5.6	.70

TABLE. 5. *Respiration in Russet Burbank potatoes at high and low temperatures. The lots consisted of 9 tubers each and ranged in weight from 1518 to 1589 g.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	At 22°C.			At 2.5 ± .5°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	10.1	11.0	1.08	3.2	3.2	1.01
2	9.9	8.9	.90	2.9	2.7	.93
3	9.4	6.5	.70	2.6	2.3	.88

TABLE 6. *Respiration of McCormick potatoes at high and low temperatures. Experiment was begun two weeks after harvest.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	At 22°C.			At 1–3°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	29.8	16.8	.56	7.1	5.7	.80
2	23.5	14.6	.62	5.3	4.1	.79
3	20.3	12.4	.61	5.8	3.0	.52

ethyl bromide gas upon both anaerobic and aerobic respiration of Russet Burbank potatoes. Four lots of five tubers each varying in weight from 841 to 880 g. were selected. Two lots were untreated; the other two were placed in a closed 12-liter desiccator with 5 cc. of ethyl bromide, which was allowed to evaporate from a wad of cotton. The tubers were exposed to the gas one hour. All lots were then placed in the respiration chambers and twice subjected to full aspirator vacuum with slow release each time. Carbon dioxide-free air or nitrogen was passed over the tubers for an additional 1.5 hours. The total

TABLE 7. *Respiration in McCormick potatoes at 1.5 ± .5°C. after varying periods of storage at the same temperature.*

Period of respiration tests	Respiration periods of 24 hours	CO_2 per kilogram per hour		
		Aerobic	Anaerobic	I/N
		mgm.	mgm.	
November 20 to 28	1	7.1	5.7	.80
	2	5.3	4.1	.79
	3	5.8	3.0	.52
January 15 to 20	1	4.9	4.5	.91
	2	4.6	4.8	1.04
	3	5.3	3.9	.73
February 20 to 25	1	3.1	3.8	1.24
	2	3.2	4.4	1.40
	3	3.6	4.4	1.20

TABLE 8. *Respiration of Russet Burbank potatoes at 30°C. before and after treatment with ethyl bromide gas.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	Control lots			Exposed 1 hr. to ethyl bromide gas		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	12.4	10.5	.85	32.0	11.8	.37
2	12.1	6.2	.51	26.6	7.8	.29
3	13.0	6.5	.50	18.7	9.7	.52
4	13.4	7.5	.56	16.9	11.4	.68

TABLE 9. *Respiration of wounded Russet Burbank potatoes at 22°C.*

Treatment before respiration tests	CO_2 per kilogram per hour Aerobic	Anaerobic	I/N
	mgm.	mgm.	
Control lot (unwounded)	12.1	6.2	.51
	13.0	6.5	.50
	13.4	7.3	.56
Wounded just before respiration tests (Lot 1)	72.5	28.6	.39
	60.1	22.8	.38
	51.6	20.5	.40
Wounded just before respiration tests (Lot 2)	47.8	17.0	.35
	42.9	14.4	.33
	35.1	10.7	.31
Stored at 2.5°C. from December 15 to January 10 (unwounded)	33.6	12.6	.37
	35.8	12.2	.34
	29.9	10.2	.34
Stored at 2.5°C. from December 16 to March 9. Wounded before respiration tests	151.8	33.8	.22
	123.9	35.1	.28
Stored at 2.5°C. from December 16 to April 7. Wounded before respiration tests	172.6	26.5	.15
	123.1	24.6	.20

period before the collections of the CO_2 was about four hours. The data given in table 8 show that the anaerobic respiration of potatoes was only slightly increased by ethyl bromide gas, although the aerobic respiration for a short time was more than two-fold greater in the treated tubers than in the controls.

Wounding.—The scant data on the effect of wounding on the anaerobic respiration of potato tubers are conflicting. Stich (1891) in one experiment found that anaerobic respiration of wounded tubers was about the same as the aerobic respiration of unwounded tubers. In another experiment he found an increase in anaerobic respiration after wounding. In both experiments, the time in hydrogen was very brief. Lutman (1926) claims that the anaerobic respiration of potatoes does not respond to wound stimulus. Potatoes were wounded by hacking with a light-weight, sharp knife. The cuts were about 3 mm. deep, and spaced a similar distance approximately. The cuts were made in two directions so that they crossed each other at right angles, or nearly so. The wounded surface was approximately four times the area of the original surface of the tuber. The respiration tests were discontinued after two or three days in order to avoid complications due to bacterial action on the wounded tubers. The respiration samples were small, consisting of three tubers each. The previous storage treatments and the results of the respiration tests are given in tables 9 and 10.

Wounding increases both the aerobic and anaerobic respiration in potatoes but the lower I/N values for the wounded potatoes indicate that wounding increases the aerobic much more than it does the anaerobic respiration. The respiration rate was very high in potatoes that were wounded immediately after being removed from cold storage. The effect of the wound stimulus was superimposed upon the effect of previous cold storage, but when the results were corrected for the latter effect, it was found that the wound stimulus, especially for the aerobic respiration, was much greater in the cold storage potatoes than in similar potatoes stored at room temperature.

CARROTS (*Daucus carota* L.).—Danvers Half-Long or closely related varieties were used in the respiration experiments with carrots. Bunched carrots were purchased as needed on the open market. They were always in good condition but not freshly harvested. The tops were cut off about one-fourth inch above the crown. Since the roots varied in size and shape, lots were sorted carefully to equalize,

TABLE 10. *Respiration of wounded and unwounded Irish Cobbler potatoes at 22°C.*

Storage treatments before wounding and respiration tests	CO_2 per kilogram per hour Control lots (unwounded) Aerobic	Anaerobic	I/N	Wounded Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
Room temperature for 47 days	5.1	6.3	1.23	73.2	14.0	.19
Stored at 2.5°C. for 56 days	28.7	12.6	.44	144.3	25.9	.18

TABLE 11. *Respiration in carrots at high and low temperatures. The lots consisted of 12 roots each and weighed from 569.5 to 581.59 g.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	28 ± .2°C.			3 — 4°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	68.2	105.5	1.54	14.1	12.5	.89
2	60.6	105.1	1.74	12.2	11.0	.90
3	52.6	100.8	1.92	12.3	10.0	.82
4	51.3	102.2	1.99	12.4	11.1	.90

TABLE 12. *Respiration in carrots at high and low temperatures. The lots consisted of 8 roots each and weighed from 486 to 496 g.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	28 ± .3°C.			3 ± .5°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	65.6	98.7	1.50	12.8	13.7	1.07
2	57.4	92.7	1.62	11.0	12.3	1.12
3	55.7	90.6	1.63	9.9	10.8	1.09
4	49.7	86.4	1.74	9.1	10.6	1.17
5	48.0	89.3	1.86	9.8	11.4	1.15

as nearly as possible, the variations in the different lots. The respiration temperatures and the results are shown in tables 11 and 12.

The experiments show a consistently high anaerobic respiration in carrots. The I/N value for the first day at the warm temperature was about 1.50 and it increased slightly as the time went on. At the cold temperature, the I/N values were about unity and remained fairly constant.

There was no visible mold at any time and there was no sign of bacterial infection. The roots in table 12 were dipped for three minutes in 50 per cent alcohol and then rinsed with sterile water. The odor and taste of the roots were quite normal after a week of anaerobiosis, and the appearance of the roots was normal. The aerobic sample at 28°C. showed a little growth of leaves at the crown but there was no growth on the anaerobic sample.

The Pasteur effect in carrot roots was first suggested by the early experiments of Richards (1896) and also by those of Boysen-Jensen (1923). Marsh and Goddard (1939) using the slice technique clearly demonstrated the Pasteur effect in carrot root tissue. The data in tables 11 and 12 obtained with whole roots confirm the very pronounced Pasteur effect in carrots.

PARSNIPS (*Pastinaca sativa*).—Parsnips of the Hollow Crown variety were brought immediately

TABLE 13. *Respiration in freshly harvested parsnips at high and low temperature.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	22 ± .3°C.			1.5 ± .5°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	53.9	78.8	1.46	20.8	21.4	1.03
2	45.5	66.7	1.47	16.6	14.2	.86
3	43.4	71.6	1.65	15.0	18.0	1.20
4	...	...	...	13.7	14.1	1.03
	After 30 days storage at 1.0° to 2.5°C.					
1	65.8	67.3	1.02	12.1	15.7	1.30
2	52.6	62.4	1.19			
				10.7[a]	12.7[a]	1.18[a]
3	49.4	72.3	1.46			

[a] Average of 2 days.

TABLE 14. *Respiration in turnips at 22 ± 0.5°C.*

24 hour periods	CO_2 per kilogram per hour					
	Fresh roots			After 17 days storage at 2.2°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	69.8	47.5	.68	90.4	50.7	.56
2	66.2	40.4	.61	86.2	39.2	.45
3	58.5	41.7	.71	74.4	36.5	.49
	...	...	...	67.3	36.9	.55

to the laboratory after digging and prepared for the experiments which were started the same day. The lots consisted of seven roots each and the weights ranged from 540 to 640 g. Four lots were used to determine the respiration in the freshly harvested parsnips. The remaining lots were placed in moist loam soil and stored for 30 days at 2.2°C. These lots were then removed for respiration tests. The weights of the stored lots remained almost unchanged during the storage period. The results of the respiration tests are presented in table 13.

The aerobic respiration at 22°C. decreased with time both in the freshly dug roots and in the roots that had been stored at low temperature. The anaerobic respiration did not decrease during the experiments, therefore the I/N values increased. Only the aerobic respiration showed the "change of temperature effect." At the low respiration temperature, the I/N values remained fairly constant. The slight top growth of the aerobic lots did not seem appreciably to affect the rate of respiration. The data in table 13 present for the first time a Pasteur effect in parsnip roots. It is almost as pronounced as that in carrot roots. In both carrots and parsnips the I/N ratios at the low temperature were less than those at the higher temperature.

TURNIPS (*Brassica rapa* L.).—The turnips used in this experiment were purchased on the local market. After removing the tops which were still fairly fresh, four lots of five roots each were selected. Respiration tests were begun immediately on two lots. The other two lots were stored for 17 days at 2.2°C. They were placed in moist chambers with loosely fitting covers, and a piece of moist towel paper placed over the roots. With this precaution, the weight of the roots remained constant. The results are shown in table 14.

The turnips of both aerobic samples developed some new root and leaf growth. There was no leaf or root growth in the anaerobic samples. Cold storage of turnips resulted in an initial increase in the aerobic respiration at subsequent higher temperature, but the anaerobic respiration seemed quite unaffected by such treatment.

ONIONS (*Allium cepa* L.).—The onions, purchased on the market, were of the Yellow Globe variety. After the loose outer scales were removed, four comparable lots of 17 onions each were selected. The lots ranged in weight from 645 to 741 g. Respiration measurements were begun immediately on two lots. The other two lots were stored for 18 days at 2.2°C. before beginning the respiration tests. The results are presented in table 15.

The aerobic respiration was slightly stimulated by the cold storage, but this was not true of the anaerobic respiration. This is indicated by the difference in the I/N values before and after cold storage.

TABLE 15. *Respiration in onions at 22°C. before and after cold storage.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	Before storage			After storage for 18 days at 2.2°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	42.0	23.4	.56	53.6	21.0	.39
2	39.3	20.0	.51	48.5	18.0	.37
3	37.3	19.3	.52	48.2	16.1	.33
		Aerobic				
4	38.2	39.7	...	47.3	17.5	.37
5	38.4	49.4	...	48.2	18.2	.38
6	38.1	49.9	...	48.6	19.2	.40
					Aerobic	
7	35.0	51.4	...	46.4	40.0	...
8	36.8	49.0	...	45.4	56.8	...
9	36.4	51.3	...	45.1	57.1	...
10	36.2	50.4	...	48.6	62.5	...

TABLE 16. *Respiration in tomatoes at different stages of ripeness.*

Respiratory periods of 24 hours	CO_2 per kilogram per hour					
	28 ± .2°C.			5.5 ± .5°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
			Green stage			
1	50.6	35.1	.69	4.1	6.8	1.68
2	53.4	33.8	.63	7.5	6.0	.80
3	47.3	31.3	.66	8.7	6.5	.75
4	40.8	...	...	7.9	5.4	.68
5	...	...	...	8.0	5.2	.65
6	...	...	...	8.4	5.1	.61
			Pink stage			
1	60.2	30.8	.51	10.3	9.9	.97
2	50.0	21.5	.43	10.6	9.2	.87
3	40.9	17.4	.43	11.0	8.1	.73
4	36.7	18.6	.51	11.0	7.6	.69
5	34.0	...	...	9.5	6.6	.70
6	...	...	...	9.4	6.2	.66
			Red ripe stage			
1	50.2	26.6	.55	7.4	5.4	.73
2	42.0	22.3	.53	8.6	6.2	.72
3	37.8	16.3	.43	8.5	5.7	.67
4	35.3	...	...	8.2	5.1	.62
5	...	...	...	8.5	5.3	.62

All the lots produced both leaf and root growth during the experiment but the anaerobic lots did not show growth until changed to air. This new growth did not seem to increase the normal respiration rate in the bulbs.

TOMATOES (*Lycopersicon esculentum*).—Gustafson (1930) measured the carbon dioxide liberated from tomato fruits at 25°C. both in air and in nitrogen. In all cases there was usually a temporary increase in CO_2 production when the fruits were deprived of oxygen by substituting nitrogen, but the anaerobic production of carbon dioxide was not the same for all stages of development. In the following experiment the I/N ratios were calculated for both a high and a low respiration temperature and for three different stages of ripeness, approximating the stages in color shown by Sando (1920). The green fruits were usually light green in color, just before the appearance of pink; pink fruits were colored pink over a part of the surface, seldom more than half; red ripe fruits were completely red but did not show signs of softening. The tomatoes used for the experiment were of the Marglobe variety. Each lot consisted of eight fruits and weighed about 950 g. The tomatoes were picked about noon and placed in the respiration chambers about 3 p.m. The results are shown in table 16.

The aerobic respiration of the fruits was about the same as reported by Gustafson at the high temperature. The fruits in the pink stage had the highest initial aerobic respiration. The respiration decreased sharply as the fruits ripened. The green tomatoes had the highest anaerobic respiration at the warm temperature, both in actual quantity of CO_2 liberated and in proportion to the aerobic respiration. At low temperature, the tomatoes in the pink stage produced the most CO_2 under both aerobic and anaerobic conditions.

At the higher temperatures, the I/N values were slightly higher for green tomatoes because of the higher anaerobic respiration in proportion to the aerobic respiration. At the low temperature, the I/N values were about the same for all stages, but they were consistently higher than those at the high tem-

TABLE 17. *Respiration of Hopeland sweet corn at 30°C.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	Milk stage			Early dough stage		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	418.1	236.4	.57	347.6	179.4	.52
2	370.4	186.2	.50	298.5	141.2	.47
3	316.4	147.6	.47	258.6	139.5	.54

TABLE 18. *Respiration of Trucker's sweet corn in the milk stage at 3 ± 1.0°C. for six days and then at 30°C.*

Respiration periods of 24 hours	CO_2 per kilogram per hour		
	Aerobic	Anaerobic	I/N
	mgm.	mgm.	
1	67.9	43.1	.63
2	57.4	41.9	.73
3	61.8	41.8	.68
4	53.0	42.4	.80
5	49.9	39.3	.79
6	52.7	39.2	.74
	Changed to 30°C.		
1	373.8	253.9	.68
2	315.9	185.0	.59
3	286.3	127.8	.45

perature. In no cases were the I/N ratios greater than unity.

GREEN SWEET CORN (ZEA MAYS L.) AT DIFFERENT STAGES OF RIPENESS.—Green sweet corn of the Hopeland variety was picked early in the morning and prepared immediately for the experiment. Respiration measurements were made on two lots of corn in the milk stage and on one lot in the early dough stage. The corn in the milk and dough stages was 19 and 26 days respectively from the time the first silks appeared. The results are given in table 17.

The average I/N values for both stages of ripeness were practically the same, indicating that the aerobic and anaerobic respiration decreased at the same rate from the milk to the dough stage.

GREEN SWEET CORN AT A HIGH AND A LOW RESPIRATION TEMPERATURE.—Experiments were conducted with two varieties of sweet corn, Trucker's and Stowell's Evergreen. The corn was in the typical milk stage and the respiration samples consisted of four ears each. The respiration temperatures and the results are given in tables 18 and 19.

At the low respiration temperature the rates of both aerobic and anaerobic respiration declined very slowly with time and the I/N values remained practically constant for the period of the experiment. When the corn was transferred to 30°C., the proportional increase in the respiration rate was about the same for both types of respiration.

In the Stowell's Evergreen corn at the higher temperature the rate of anaerobic respiration declined with time more rapidly than the rate of aerobic respiration, resulting in a gradual decrease in I/N values. At the low temperature the I/N values remained almost constant. Under aerobic conditions the corn kernels shrunk and the endosperm became more doughy. Green sweet corn has an unusually high respiration rate and at high temperature it is very sensitive to anaerobic conditions. The anaerobic respiration declined rapidly at the high temperature and the corn assumed an unnatural odor although it appeared fresh and plump.

MATURED SWEET CORN AND REID'S YELLOW DENT CORN CONTINUING DIFFERENT PERCENTAGES OF IMBIBED WATER.—Bailey (1921) found that moisture content in large measure determines the rate of normal aerobic respiration of sound corn stored under uniform temperature and other conditions. The purpose of this experiment was to test the effect of different percentages of imbibed water on the anaerobic respiration as well as the aerobic respiration of mature corn. Distilled water was added directly to the shelled corn in sufficient quantity to raise the moisture content to the percentage desired. The material was stirred frequently for the first few hours to keep the water uniformly distributed over the surfaces of the kernels until it had all been imbibed. After adding the water, two or three days were allowed for it to distribute evenly throughout the kernels. During this time the corn was stored in loosely stoppered jars.

The samples of mature sweet corn of the Hopeland variety were prepared by shelling three rows from each of 50 ears. The respiration samples weighed 200 g. each. The samples of mature Reid's Yellow Dent corn were prepared in the same manner. The samples weighed 250 g. each. The respiration data are calculated on basis of dry weight. The results are presented in table 20.

The experiment shows conclusively that an increase in the imbibed water up to 22 per cent produces a great increase in the anaerobic respiration as well as the aerobic respiration in both dent corn and sweet corn. The difference in the I/N values with the different moisture contents shows that the proportional increase was actually greater in the anaerobic respiration.

TABLE 19. *Respiration rates of Stowell's Evergreen sweet corn at high and low temperatures.*

Respiration periods of 24 hours	CO_2 per kilogram per hour					
	At 27.8 ± .2°C.			At 4.5 ± 0.5°C.		
	Aerobic	Anaerobic	I/N	Aerobic	Anaerobic	I/N
	mgm.	mgm.		mgm.	mgm.	
1	326.9	169.8	.52	68.3	37.8	.55
2	297.8	115.0	.39	67.3	38.5	.57
3	271.6	93.8	.35	53.3	33.6	.63
4	256.7	76.7	.30	49.5	29.6	.60
5	229.1	....	...	51.4	34.5	.67
6	208.2	....	...	47.2	31.0	.65

TABLE 20. *Respiration in mature corn at 22°C. after imbibing different percentages of water.*

Material	Moisture	CO_2 per 100 grams dry weight per 24 hours		I/N
		Aerobic	Anaerobic	
	per cent	mgm.	mgm.	
Hopeland sweet corn	15.11	11.30	3.00	.26
	20.58	55.18	29.75	.54
Reid's Yellow Dent	17.38	12.29	3.55	.29
	22.09	66.79	38.98	.58

SUMMARY

Both aerobic and anaerobic respiration were determined in a number of different storage organs, chiefly vegetables, under a wide variety of circumstances. At the respiration temperature of 22°C., the anaerobic respiration either remained fairly constant for the short experimental period or declined at the same rate as the aerobic respiration.

Any pronounced alteration in rate of aerobic respiration was accompanied by an alteration in the same direction of anaerobic respiration, but only in a few cases were both types of respiration affected to the same degree. The most pronounced acceleration of aerobic respiration occurred for a time in potatoes after cold storage, after wounding and after treatment with ethyl bromide gas. The anaerobic respiration was very much less affected by these treatments. An increase of imbibed water in corn caused a great increase in both aerobic and anaerobic respiration.

The ratios of anaerobic to aerobic respiration in carrots, parsnips, tomatoes and McCormick potatoes had different values at high and low respiration temperatures. These ratios had about the same value at high and low respiration temperature only in Russet Burbank potatoes and in sweet corn. The ratios of anaerobic to aerobic respiration remained fairly constant during the decline in respiratory intensity in potatoes immediately after harvest and also in sweet corn at different stages of maturity and during the decline in respiration with time of storage.

The quotient CO_2 output in nitrogen/CO_2 output in air is expressed as I/N. This ratio closely approximated the theoretical value of .33 for alcoholic fermentation and aerobic respiration in the following: green sweet corn at 27°C.; Russet Burbank potatoes and onions during the initial period of high respiration after cold storage; and potatoes after wounding and after treatment with ethyl bromide gas. The I/N ratios were greater than unity at 22°C. only in carrot and parsnip roots and in McCormick potatoes. The I/N ratios closely approached unity in Irish Cobbler and Russet Burbank potatoes under most of the experimental conditions, and in tomatoes at different stages of ripening and at a respiration temperature of 5.5°C. All the other plant organs under the different experimental conditions gave I/N ratios much below unity, but somewhat greater than .33.

The Pasteur effect was very pronounced in carrot and parsnip roots and in McCormick potatoes. In some cases the quantity of CO_2 liberated by carrot and parsnip roots in nitrogen was nearly twice that liberated in air. The results with whole carrot roots confirm Marsh and Goddard's results on slices of carrot root. The pronounced Pasteur effect exhibited by parsnip roots and by a variety of Irish potato has not been previously reported. Carrot and parsnip root tissue should prove favorable material for the study of the mechanism of the Pasteur effect because of its magnitude in these tissues.

The Pasteur effect which is the basis for Blackman's theory of oxidative anabolism in his general scheme of the respiratory process in higher plants has been reported in several plant organs, but it does not appear to be of universal occurrence in higher plants.

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THE INFLUENCE OF KERNEL SIZE, AGE, LOCATION IN PANICLE, AND VARIETY OF OAT, ON THE VARIABILITY OF THE AVENA TEST [1]

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IT IS recognized by workers in the field of plant hormone research that the *Avena* test is subject to serious fluctuations. Even when known concentrations of pure chemicals are being tested, there may be considerable variation in the amount of curvature of different *Avena* coleoptiles in the same rack. The literature contains innumerable examples of this variability which need not be cited here. Went and Thimann (1937) gave a discussion of the early literature on the variability of the *Avena* test. Link, Eggers, and Moulton (1940) summarized the situation as follows: "Even with pure indoleacetic acid solution, some coleoptiles on the same day, and whole sets of coleoptiles from day to day, show curvatures more than twice those obtained on others tested with the same concentration. Reasonably good proportionalities are obtained for activity and concentration of indoleacetic acid only by averaging the results of many tests."

The experiments of Goodwin (1939), Avery, Creighton, and Hock (1939), Funke (1939), and Linser (1939) indicate that this variability in *Avena* coleoptile curvature occurs in all of the commonly used modifications of the *Avena* test, such as Went's "standard," Skoog's "deseeded," Funke's coleoptile section, and lanolin paste methods. Possibly even more serious than the variability of *Avena* curvatures is the fact that many people who read the reports of phytohormone research do not carry on tests themselves and do not appreciate the fluctuations associated with the *Avena* technique. The criticism of the *Avena* test is based on its variability and not on a lack of sensitivity.

The experiments being reported here were designed to investigate a number of factors related to the oat kernel or variety which might influence the fluctuations in the *Avena* test, and possibly determine techniques which would eliminate some of these variations.

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METHODS AND MATERIALS.—Skoog's (1937) "deseeded" *Avena* test method was used for auxin assays. The agar containing the auxin was always applied to the decapitated coleoptiles at between 10:00 and 11:00 a.m. to overcome any possible effect of diural fluctuations. The Victory variety of oats (secured from M. Ramsey, Batavia, N. Y.) was used in all tests unless otherwise indicated.

RELATION OF SIZE OF AVENA KERNEL TO CURVATURE.—A series of tests was conducted to determine if the size of the oat kernels might influence the amount of curvature secured during hormone tests. A large number of individual grains of Victory oats were weighed on an analytical balance and were found to vary from approximately 0.01 gram to 0.04 gram each. For testing purposes the kernels were divided into three size groups: (1) less than 0.02 gram, (2) 0.02 to 0.03 gram, and (3) over 0.03 gram. In these tests indoleacetic acid was used as the test solution at a concentration of 20 micrograms per liter.

The data secured in these tests are presented in table 1. If the average curvatures for the entire series are considered, it will be seen that the curvature of coleoptiles from small kernels is 0.7 degrees greater than that of coleoptiles from kernels of medium size, which, in turn, is 1.7 degrees greater than curvatures of coleoptiles from large kernels.

From the standpoint of practical application, the above recorded differences are of minor importance. The greatest differences in average curvature between tests in the same size group amount to 4.3 to 5.0 degrees, which is more than the average difference between the size groups. In other words, the greatest and smallest curvatures within the size groups varied from 19.0 to 21.6 per cent from the average of the group, whereas the deviation of the

individual groups from the average of all groups taken collectively was from 2.5 to 11.5 per cent. There is, however, more uniformity between kernels of the same size than between all sizes taken collectively.

TABLE 1. *Effect of size of oat kernels on the curvature of Avena coleoptiles used in plant hormone tests. Test agar contained 20 micrograms per liter of indoleacetic acid. The oats used in these tests were from North Dakota and were less sensitive to the unilateral application of auxin than the Victory oats from Ramsey of Batavia, New York. Each figure represents the average curvature of 12 coleoptiles.*

Date of test	Size of kernels		
	0.02 gram	0.02 to 0.03 gram	0.03 gram
10/31	14.1	14.2	10.6
10/31	14.8	13.5	9.3
11/1	15.4	13.3	11.1
11/1	15.6	11.1	12.4
11/2	13.6	12.6	10.9
11/2	10.7	13.8	11.3
11/2	12.5	11.9	10.6
11/4	12.8	10.7	12.2
11/4	13.3	13.1	9.7
11/4	11.9	11.3	12.1
11/5	13.3	12.3	10.8
11/5	14.5	13.6	11.0
11/5	11.3	12.7	12.0
11/6	11.8	14.2	10.0
11/6	13.7	10.6	9.5
11/6	12.2	13.6	11.7
3/3	12.8	12.0	11.9
3/3	12.1	12.4	12.1
3/3	15.0	13.3	9.5
3/3	14.4	14.0	10.5
3/4	12.9	11.9	10.9
3/4	11.0	10.7	9.6
3/4	12.3	12.1	11.2
3/5	11.7	11.0	8.5
3/5	12.9	11.5	11.2
3/5	14.9	15.0	12.8
3/6	13.5	12.1	8.6
3/6	12.0	14.0	9.6
3/6	12.6	13.0	12.0
3/7	14.2	12.6	10.9
3/7	13.0	12.2	11.2
3/7	13.4	10.0	9.3
Average	13.2	12.5	10.8

For the benefit of those readers who have not actually conducted *Avena* tests, it might be stated that the above curvatures represent rather uniform results. Curvatures of coleoptiles treated with extracted auxin frequently fluctuate to a much greater degree.

In connection with the above described tests, it was found that the yield of usable test plants was higher from a given number of small- and medium-sized grains than from a given number of large grains. The coleoptiles from the small- and medium-sized kernels were smaller in diameter and longer than those from large kernels and were easier to decapitate and use in the hormone test. The Marketon variety of oat, which is sometimes used in the *Avena* test, exhibited similar variations in curvature of coleoptiles from kernels of different sizes.

The conclusion to be drawn from the above tests is that the size of the oat kernels used in obtaining coleoptiles for plant hormone research has a minor effect on the variability of the results. It is suggested, however, that large grains be discarded, since slightly higher and more uniform curvatures are obtained from the coleoptiles from small- and medium-sized grains, and the coleoptiles also are of somewhat more uniform size.

CURVATURE OF AVENA COLEOPTILES FROM SEED COLLECTED FROM DIFFERENT POSITIONS IN THE PANICLE.—Thimann (1934) and van Overbeek (1938 and 1940) have demonstrated that auxin in *Avena* coleoptiles and some other plants is more concentrated in apical portions than in basal portions. It appears, therefore, that coleoptiles from kernels collected from different positions in the oat panicle may differ in auxin content and, consequently, vary in sensitivity to auxin when used in the *Avena* test. A series of tests to secure information on this question was conducted using kernels collected from panicles of Swedish Select oats.

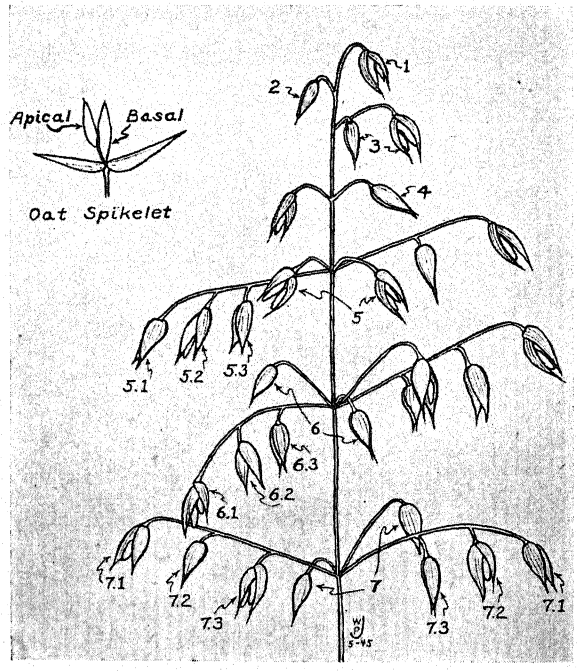


Fig. 1. Oat panicle and spikelet. Numbers indicate the locations of the kernels used in *Avena* tests.

Figure 1 illustrates a typical oat panicle and the positions from which the kernels were taken. Positions designated by whole numbers refer to spikelets which occur singly at the first, second, third, fourth, fifth, sixth and seventh nodes, numbered from the apex to the base of the panicle. The decimal num-

bers 5.1, 5.2, 5.3; 6.1, 6.2, 6.3; and 7.1, 7.2 and 7.3 refer to spikelets which occur on lateral branches at the fifth, sixth and seventh nodes. Only those spikelets which occurred singly or in groups of three on lateral branches were used in the present experiment.

Each individual spikelet contains two kernels, as illustrated in the insert of figure 1. These kernels were tested separately. The smaller terminal grain was referred to as the *apical* kernel; whereas the larger one was called the *basal* kernel because of their relative positions in the spikelet.

In these experiments, over 3,000 seedlings were grown from kernels from the 32 above-enumerated positions. Coleoptiles from kernels from each position were tested on several different days with 10 and 20 micrograms per liter of indoleacetic acid in the agar. The results were tabulated and a summary is presented in table 2.

TABLE 2. *Summary of tests with Avena coleoptiles from seeds from different locations in the panicle and spikelet. For description of locations in panicle see figure 1.*

Position of of spikelet in panicle	Average curvature: Apical kernel of spikelet	Basal kernel of spikelet	Difference between apical and basal kernels
10 micrograms per liter indoleacetic acid in test agar			
1	11.2	9.6	1.6
2	11.9	10.1	1.8
3	11.6	10.0	1.6
4	11.1	9.6	1.5
5	11.4	9.8	1.6
6	11.7	9.6	2.1
7	11.0	9.6	1.4
	Average 11.5	Average 9.8	Average 1.7
5.1	10.5	7.8	2.7
5.3	10.6	8.5	2.1
6.1	9.7	7.9	1.8
6.3	10.9	8.4	2.5
7.1	10.2	10.2	0.0
7.3	10.5	7.6	2.9
	Average 10.4	Average 8.4	Average 2.0
20 micrograms per liter indoleacetic acid in test agar			
1	18.9	15.2	3.7
2	19.4	17.4	2.0
3	19.9	17.5	2.4
4	22.6	20.1	2.5
5	21.3	19.4	1.9
6	22.4	19.2	3.2
	Average 21.1	Average 18.5	Average 2.6
5.1	22.6	20.5	2.1
5.3	19.9	17.9	2.0
6.1	24.5	20.9	3.6
6.3	24.7	22.0	2.7
7.1	25.3	22.9	2.4
7.3	25.2	22.6	2.6
	Average 23.9	Average 21.3	Average 2.6

In general, the data of table 2 indicate relatively insignificant differences in the sensitivity to auxin of coleoptiles from kernels from various positions in the panicle. In the tests with agar containing 20 micrograms per liter indoleacetic acid, the coleoptiles from kernels originating at the lower part of the panicle made somewhat greater curvatures than those from kernels originating in the upper part of the panicle. This variation in sensitivity is of minor importance, however, in consideration of the magnitude of the fluctuations commonly encountered in *Avena* tests.

TABLE 3. *Effect of the age of oat seed on the Avena test. Test agar contained 20 micrograms per liter of indoleacetic acid. The oats used in these tests were from North Dakota and were less responsive to the unilateral application of auxin than the Victory oats from Ramsey of Batavia, New York. Each figure represents average curvature of 12 coleoptiles in test rack.*

Date	9-month-old seed	21-month-old seed
5/8	9.4	11.0
5/8	13.4	9.8
5/8	14.8	11.0
5/8	14.0	13.4
5/9	11.0	10.6
5/9	9.0	9.6
5/9	10.0	9.2
5/12	12.6	12.6
5/12	13.8	10.6
5/12	12.4	9.6
5/12	9.8	10.8
5/14	9.2	12.8
5/14	12.6	9.8
5/14	11.0	9.4
5/14	9.0	11.2
Average	11.5	10.8

Date	12-month-old seed	24-month-old seed
8/23	9.8	9.6
8/23	10.4	11.4
8/24	12.7	12.6
8/24	14.5	11.2
Average	11.8	11.2

The data of table 2 indicate that coleoptiles from small apical kernels of the spikelet make an average of 1.7 to 2.6 degrees more curvature than coleoptiles from the larger basal grains. The apical grains averaged 0.017 gram in weight, while the basal kernels averaged 0.025 gram. These data support the previously recorded results with small and large Victory oats and confirm the conclusion that large grains might well be eliminated in order that more uniform curvatures may be secured in the *Avena* test.

EFFECT OF AGE OF SEED ON CURVATURE.—Funke (1939), Linser (1939), and Sweeney and Thimann (1938) have presented data which show that *Avena* coleoptile sensitivity and protoplasmic streaming vary during different seasons of the year. Since all oat seedlings used in plant hormone tests grow under conditions where the light, temperature and

humidity are always the same, it has been inferred that the age of the oat seed may be an important factor underlying the seasonal fluctuation obtained in the *Avena* tests. To check on this inference, two series of tests were conducted, one in May and another in August. The oats used in these tests were from North Dakota and the coleoptiles were less sensitive to auxin than those of the Victory oats from New York.

The results of the kernel-age tests are given in table 3 and indicate no significant variations in coleoptile curvature between oat grains 9, 12, 21 and 24 months old. Although no recently harvested seed was available for use in these tests, it seems doubtful if age of oat seed would normally be a factor in effecting the fluctuations of the *Avena* test.

TABLE 4. *Variation between different varieties of oats when used as hormone test plants. Test agar contained 10 micrograms per liter of indoleacetic acid. Each figure represents the average curvature of at least four dozen test plants on four different days.*

Variety of oats	Degrees curvature	Variety of oats	Degrees curvature
Early varieties:		Late varieties:	
Boone	3.3	Anthony	7.2
Marion	3.3	20th century	6.8
Iogold	4.1	White Russian	7.2
Midseason:		Victory (N. Dak.)	5.7
Rainbow	4.9	Victory (Idaho)	7.3
Rusota	6.2	Victory (N. Y.)	8.5
Vanguard	4.2		

VARIATION BETWEEN DIFFERENT VARIETIES OF OATS WHEN USED AS HORMONE TEST PLANTS.—Due to the lower sensitivity of the coleoptiles of a North Dakota supply of Victory oats as compared to a supply from New York, it was decided to conduct tests on a number of varieties to determine their relative value as test plants. Twenty-two varieties of early-, midseason- and late-maturing oats were secured from the U. S. Department of Agriculture for use in these tests.

All of the varieties exhibited the fluctuations in curvature which have been discussed in connection with the Victory strain. A summary of the data from a number of the more common varieties is included in table 4, which shows that the late maturing types are more sensitive in the *Avena* test than are the early and midseason strains. The early varieties yielded the most straight uniform plants per 100 seeds. The late varieties, however, were not only more sensitive in the *Avena* test but the primary leaf was easier to withdraw when the plants were decapitated. The Victory variety, particularly the New York strain, falls into the group of more sensitive varieties.

SUMMARY

The experiments reported in this article were undertaken in an attempt to determine the effect of kernel size, age, location in the panicle, and variety of oat on the fluctuations in coleoptile curvature which commonly occur in the *Avena* test. All conclusions apply specifically to the Skoog (1937) "deseeded" method. No factors were discovered which would account for the marked variations in curvature, but the following conclusions are of interest and have at least a minor influence on the uniformity of *Avena* test results.

Coleoptiles growing from small-sized kernels of Victory oats are somewhat more sensitive to the unilateral application of auxin than are the coleoptiles from large kernels.

Coleoptiles from comparable kernels of spikelets from different parts of the panicle exhibit similar sensitivity in the *Avena* test. Coleoptiles from the small apical kernels of the spikelet make greater curvatures in the *Avena* test than those from the large basal grains. This substantiates the data secured from the mechanical sorting of a random sample of oats.

The age of oat seed (from 9 to 24 months) has no significant influence on sensitivity to auxin of coleoptiles produced from such kernels.

Coleoptiles of early-maturing varieties of oats are less sensitive in the hormone test than are those of late varieties. The Victory oat which is commonly used in plant hormone work is a late-maturing variety.

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THE MORPHOLOGICAL VARIATIONS AND THE OCCURRENCE OF ANEUPLOIDS IN SOME SPECIES OF AGROSTIS IN RHODE ISLAND [1]

Irene H. Stuckey and William G. Banfield

THE BENT grasses have been widely cultivated and used in natural stands in Europe and the United States for more than 200 years, and it is now difficult to determine where they are native and where introduced. Although some species of this group of *Agrostis* appear to be indigenous to North America (Hitchcock, 1905, 1920, 1935), *Agrostis alba* L. was undoubtedly introduced from Europe and so was *A. tenuis* Sibth. except, perhaps, in the northern part of its range. The differentiation of the *alba* complex into species has been equally difficult for taxonomists in Europe and in America. In this paper, Hitchcock (1935) has been used as a standard reference of nomenclature and no attempt has been made to integrate the European taxonomy.

Hegi (1906) divided the group into two species, *Agrostis alba* L. and *A. vulgaris* With., the latter being the same as *A. tenuis* Sibth. in Hitchcock's manual. Under each of these, he listed a number of varieties including as varieties of *A. alba* L., var. *prorepens* Aschers. (*A. stolonifera* L.) and var. *maritima* Meyer (*A. palustris* Huds.). Philipson (1937) recognized three species: *A. gigantea* Roth. (*A. alba* L.) with two varieties; *A. stolonifera* L. with two varieties and several forms; and *A. tenuis* Sibth. with two varieties. Jansen and Wachter (1940) used similar treatment but recognized only two species, *A. stolonifera* L. and *A. tenuis* Sibth. According to Junge (1913), all variations should be considered as forms of the *alba-stolonifera* complex. In Canada, Malte (1928) believed the *alba-palustris* complex to be varieties of *A. stolonifera* L. and *A. tenuis* Sibth., a distinct species containing two varieties.

On the basis of ligule differences Armstrong (1937) separated the group into two species, *A. alba* L. and *A. tenuis* Sibth., with the variations as varieties under *A. alba*. He considered *A. alba* to be polymorphous and recognized intergrades as well as distinct varieties within the species. Shishkin (1935) divided the bent grasses occurring in Russia into several species but suggested that it would be less confusing to reject *A. alba* L. and to divide *A. gigantea* Roth. into several races. Fouillade (1932) found specimens in France showing all degrees of variation between *A. alba* and *A. vulgaris*. He believed these plants to be hybrids and referred the specimens to Häckel, quoting part of the correspondence in his paper: " 'The differentiation of *A. vulgaris* and *A. alba* is not always so easy as the diagnosis in the book would lead one to believe; while a single character alone is not enough to separate them, a group of these characters will enable them to be distinguished in most instances. A sharp differentiation is not always possible and transition forms occasionally exist.' " He was of the opinion that " '*A. vulgaris* should be regarded as a subspecies of *A. alba*.' "

Hitchcock (1935) in this section of *Agrostis* lists: *A. stolonifera* L., *A. palustris* Huds., *A. nigra* With., *A. alba* L. and *A. tenuis* Sibth. *Agrostis stolonifera* L. appears to be native to North America and *A. tenuis* var. *aristata* (Parn.) Druce is probably native at least in the more northern part of its range. The first four species listed tend to intergrade (p. 331) but apparently *A. tenuis* is considered a species distinct from these first four, although in an earlier study (1905), *A. alba vulgaris* (With.) Thurb. was described as a subspecies of *A. alba,* and *A. alba aristata* Gray, a form of *A. alba vulgaris*. In describing the subspecies, Hitchcock states: "This subspecies can be separated only arbitrarily from the species as there are so many intermediate forms . . . . The descriptions of the European plant state that the panicle remains spreading after flowering, while in *A. alba* the panicle contracts. I have not observed this to be a diagnostic character. The form here described is common in New England and less so farther south and seems fairly distinct through this region. But in the Western States, especially in the mountain region, the forms intergrade causing great perplexity." This intergradation of *A. alba* and *A. vulgaris* (*A. tenuis* in the 1935 edition) is not mentioned in the 1935 manual.

Since these divergent opinions as to what constitutes the species have been recognized, the chromosome numbers found in the literature are reported according to the species which the plant was called by the author and no attempt has been made to determine which system of nomenclature was used. Several chromosome counts for *Agrostis tenuis* Sibth. (*A. vulgaris* With.) and other closely related species have been recorded, but they are, with the exception of those of Nielsen and Humphrey (1937) and Church (1936), entirely from European sources.

Agrostis alba L. was reported as having a 2n chromosome number of 42 by Avdulov (1931) and by Skovsted (1939) from Russia. The number in the same species was found to be 28 by Sokolovskaya (1938) also in Russia and by Nielsen and Humphrey (1937) in the United States, although the camera lucida drawing used by the latter in illustrating this

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Acknowledgment is made to Drs. Helen Hill and W. M. Myers for assistance and advice throughout, to Mr. J. R. Swallen for some of the European taxonomy and to Dr. Stephen Pieniazek for the Russian translations. Part of the data used here were taken from the thesis presented by the junior author to Rode Island State College in partial fulfillment of the requirements for the degree of Master of Science.

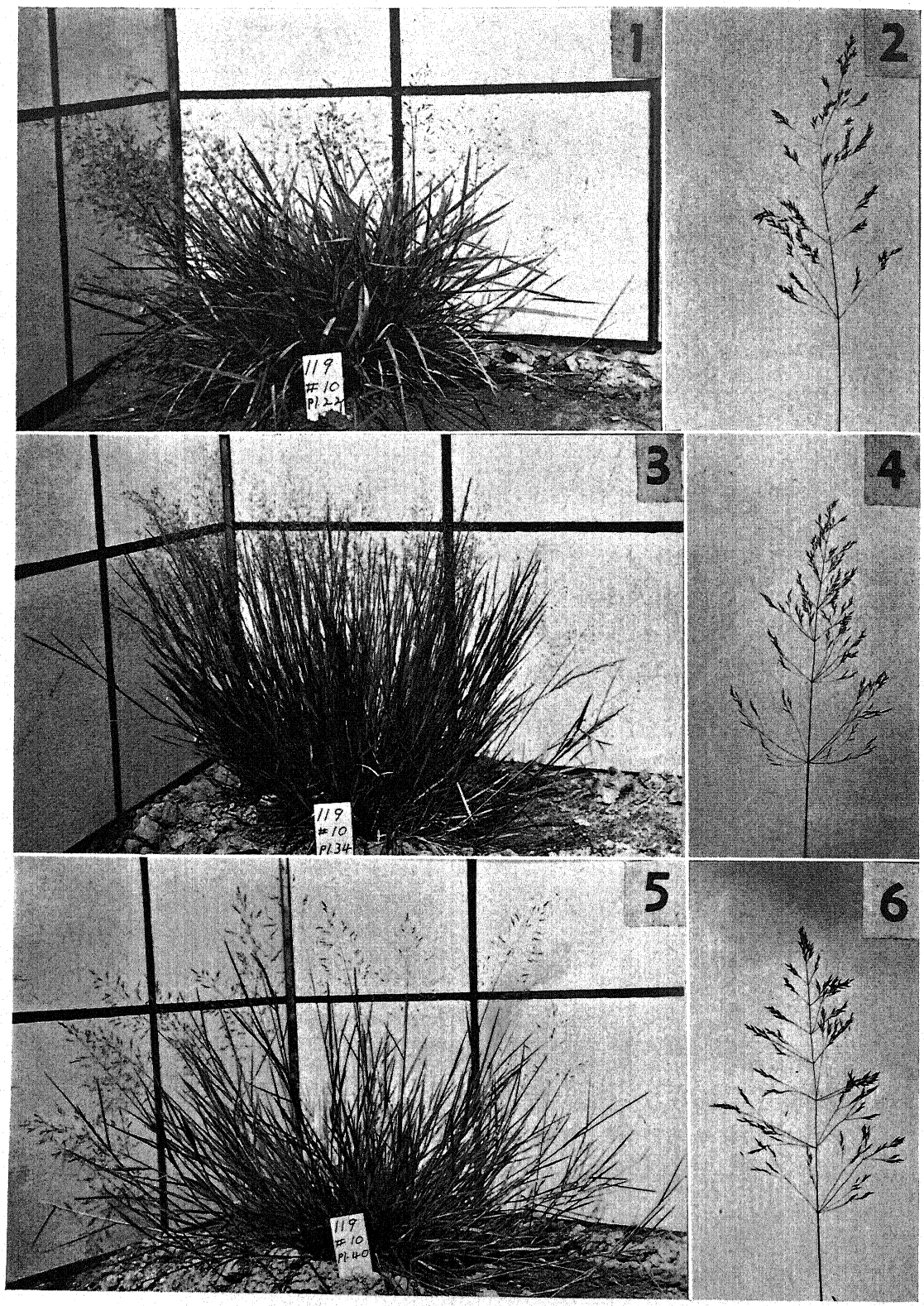


Fig. 1–6.—Selected plants from the progeny of line 119–10.—Fig. 1. Plant 119–10–22, *tenuis* type.—Fig. 2. Panicle from plant 119–10–22.—Fig. 3. Plant 119–10–34, *tenuis* type.—Fig. 4. Panicle from plant 119–10–34.—Fig. 5. Plant 119–10–40, *tenuis* type.—Fig. 6. Panicle from plant 119–10–40. In these and the following photographs, the size of the

species shows 42. *Agrostis prorepens* G. May. and *A. gigantea* Roth., which are considered by Ascherson and Graebner (1902) to be varieties of *A. alba* L., are reported as 35 and 42 respectively by Sokolovskaya (1938). In *A. nigra* (With?) a count of 42 was reported from England by Maude (1940). Church (1936) found a chromosome number of 28 in *A. palustris* Huds., but apparently this nomenclature was based on Hitchcock (1920) who gave this name to plants called *A. alba* L. in the 1935 manual. Church (1936) reported a count of 56 for *A. alba* var. *maritima* (Lam.) Meyer, presumably *A. palustris* Huds.

MATERIALS AND METHODS.—In 1940, a bent grass breeding program was begun in Rhode Island. Seed heads were collected by several staff members from plants which were presumably Colonial bent, *Agrostis tenuis* Sibth. from old pastures and fields in Rhode Island and adjacent Massachusetts and Connecticut. At the time of collection, the identity of the plants was established on the basis of the short truncate ligule and more delicate habit as distinguished from the pointed ligule and coarse plant of redtop, *Agrostis alba* L. The seedlings were grown in the greenhouse during the winter of 1940–41 and transplanted to the field in the spring. In all, there were approximately 4000 plants representing 758 lines.

Chromosome counts from root tips were made for some of these plants. The earlier counts were made from slides stained with iron-alum haematoxylin or with crystal violet and the most recent ones according to the method developed by Hill and Myers (1945). A number of the slides used in this study were made by the authors, some by Dr. Helen Hill and some by Dr. E. A. Palmatier. Each count listed is that taken from sections of at least three different root-tips.

These grasses when self-pollinated were very largely sterile but most of the open-pollinated plants produced an abundance of highly fertile seed. In July, 1944, when seeds were collected from 134 open pollinated plants, the amounts of cleaned seed per plant varied from a trace to eleven grams, and only three lots of seed showed poor germination. The notes recorded in this paper are based almost entirely on observations of open-pollinated plants although some selfed material was available.

OBSERVATIONS AND DISCUSSION.—Ten per cent of the progenies were obviously *A. tenuis* and more or less uniform but the remainder showed all degrees of variation. Some of these deviations undoubtedly came well within allowable species variation, others were intermediate forms and the most variable progenies contained plants which could not possibly be identified as *A. tenuis* along with plants which were *A. tenuis* in all respects. Most of these extreme forms resembled *A. alba* but a few showed some characteristics of *A. palustris* Huds. as well.

In that large percentage of the progenies showing a greater or lesser degree of variation, two types of intermediate plants were observed. Some plants were *A. tenuis* in all respects except for one or two characters, for example, a long pointed ligule or dense basal branches on the panicle, or a panicle pyramidal in shape. Other plants showed a blending of one or more characters, perhaps a short pointed ligule, or narrow leaves with harsh texture or a dense oblong panicle (fig. 1–18).

TABLE 1. *2n chromosome counts and plant variation in Agrostis.*

Plant number	2n count	Plant type	Variation of line
12–9	30	tenuis	extremely variable
13–2	34	tenuis	extremely variable
13–3	37	intermediate	extremely variable
20–9	30	tenuis	variable
47–1	33	tenuis	variable
94–1	39	alba	extremely variable
120–6	41	tenuis	variable
129–9	30 or 31	tenuis	variable
138–6	28	tenuis	uniform tenuis
158–10	28	tenuis	uniform tenuis
162–8	28	tenuis	variable
232–2	32	intermediate	uniform tenuis
338–6	28	dwarf tenuis	uniform tenuis
338–8	30	tenuis	uniform tenuis
455–1	31	intermediate	extremely variable
648–7	29	tenuis	uniform tenuis
659–2	30	tenuis	uniform tenuis
668–2	34	tenuis	uniform tenuis
678–8	35	tenuis	extremely variable
746–8	28	intermediate	uniform tenuis
RI 34–1–14 inbred	35	tenuis	uniform tenuis
39 F 1–2 (Penn)	42	alba	.....
119–7	34	tenuis	extremely variable
119–10–17	35	tenuis	↓
119–10–22	35	tenuis	↓
119–10–26	31	tenuis	↓
119–10–34	31	tenuis	↓
119–10–40	33	tenuis	↓
130–1–17	39	alba	extremely variable
130–1–25	35	intermediate	↓
130–2	42	alba	↓
130–2–1	39	alba	↓
130–2–14	35	alba	↓
130–2–23	37	intermediate	↓
130–2–24	36	alba	↓
130–3–6	33	alba	↓
130–3–12	33	intermediate	↓
130–8	37	intermediate	↓
316–2	31	tenuis	extremely variable
316–2–1	36	intermediate	↓
316–2–4	31	tenuis	↓
316–2–5	34	intermediate	↓
316–2–7	28	tenuis	↓
316–2–8	29	intermediate	↓
316–7	30	intermediate	↓

plants may be estimated from the 12-inch squares painted on the background. All the panicles were photographed at the same magnification, being about ½ natural size. (Photographs by George F. Gee.)

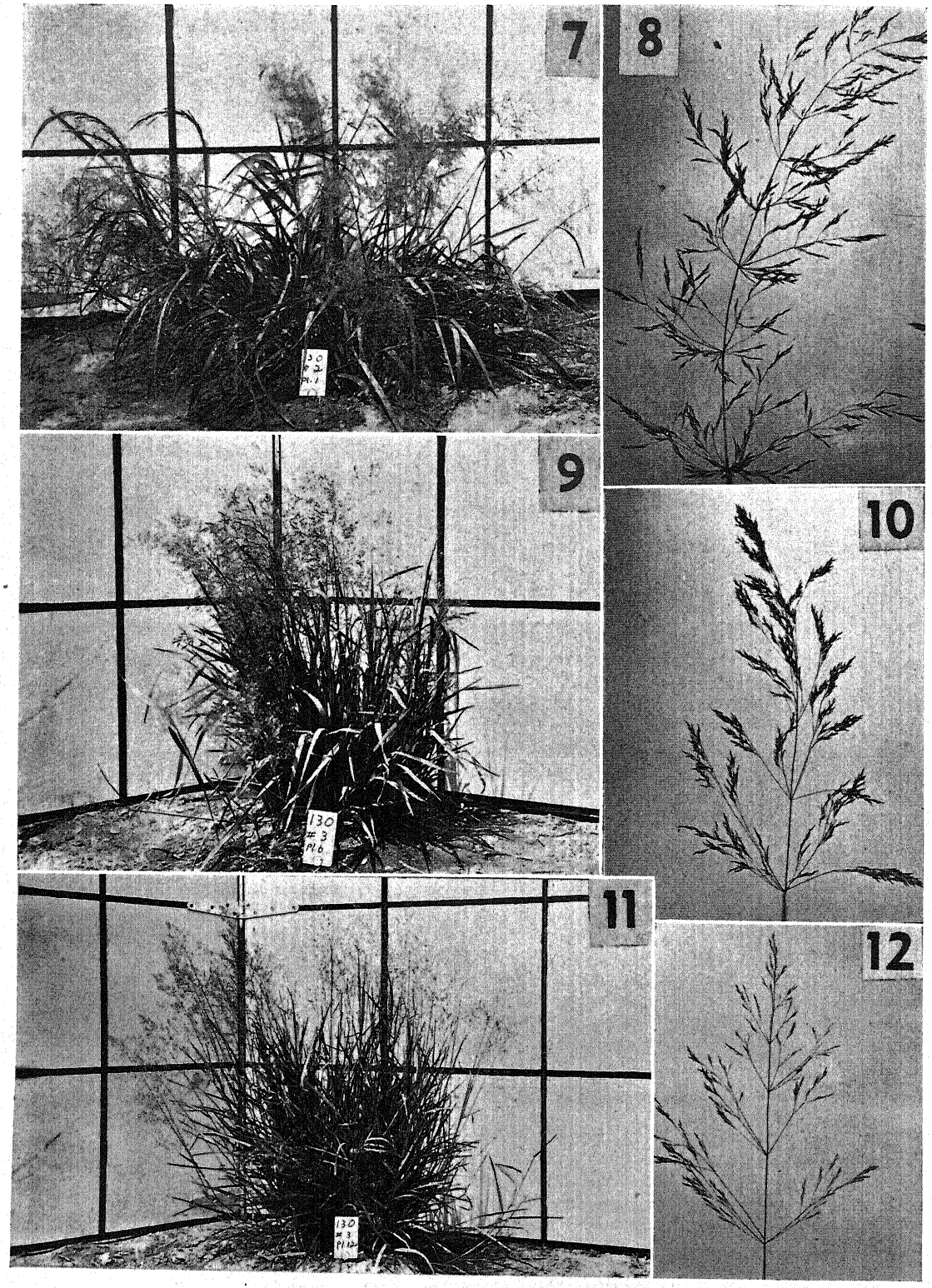


Fig. 7–12.—Selected plants from the progeny of Line 130.—Fig. 7. Plant 130–2–1 *alba* type.—Fig. 8. Panicle from plant 130–2–1.—Fig. 9. Plant 130–3–6 *alba* type.—Fig. 10. Panicle from plant 130–3–6.—Fig. 11. Plant 130–2–12 intermediate type.—Fig. 12. Panicle from plant 130–3–12.

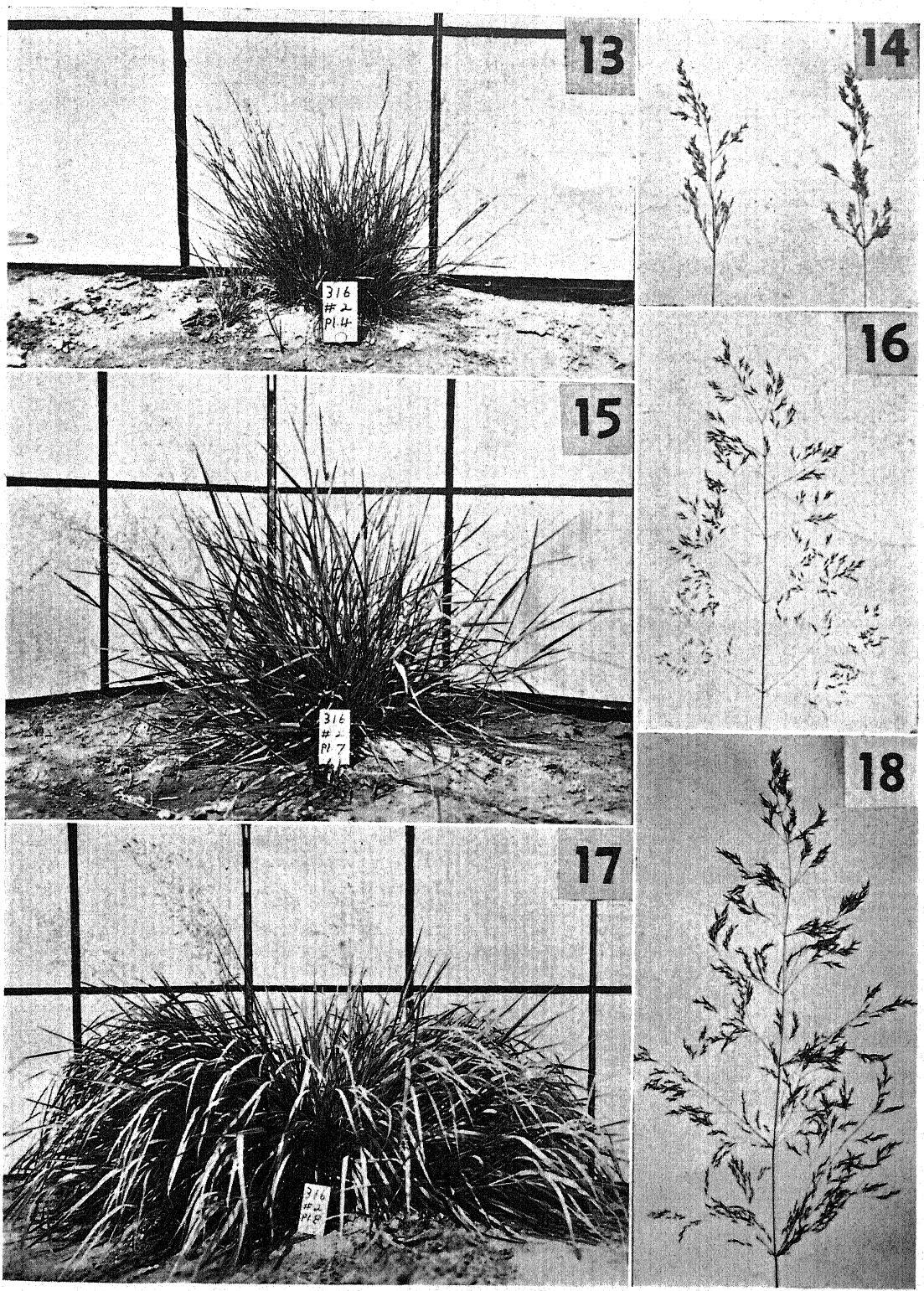


Fig. 13–18.—Selected plants from the progeny of Line 316–2.—Fig. 13. Plant 316–2–4 *tenuis* type.—Fig. 14. Panicle from plant 316–2–4.—Fig. 15. Plant 316–2–7 *tenuis* type.—Fig. 16. Panicle from plant 316–2–7.—Fig. 17. Plant 316–2–8 intermediate type.—Fig. 18. Panicle from plant 316–2–8.

During the summer of 1944, the lines remaining in the nursery and their progenies were further checked for deviation from the species type. Of these, 19 lines showed extreme variation, 85 were somewhat variable and 94 lines were classified as uniform *tenuis,* although at times it was difficult to decide how much deviation was permissible within species limits and which lines showed "variation." If these same progenies were reclassified another year, undoubtedly some lines in the last two groups would be interchanged.

From the data available in table 1, the plants of the *alba* type tend to have chromosome numbers above 35 and those of the *tenuis* type numbers below 35, but there are many exceptions. Plant 120–6 is a *tenuis* type with 41 chromosomes and 130–3–6 an *alba* type with 33. The intermediate chromosome number 35 may be found in an intermediate plant 130–1–25, an *alba* plant 130–2–14 or a *tenuis* plant 119–10–17. Hence at present, there appears to be no real evidence that there is any correlation between chromosome number and plant morphology.

Line 119 had some unusual features. All of the progenies examined contained from 19 to 40 plants. The original #119 plants showed considerable variation. In the second generation the progeny of 119–5 contained all variations from *A. tenuis* to *A. alba,* the parent plant 119–5 being more like *A. alba.* The progenies of 119–6 and 119–9 show some variation but all well within the species range except for the presence of longer and pointed ligules on several plants, while the progenies of 119–7 and 119–10 are classic *A. tenuis* in all respects. Yet the chromosome number of this last progeny seems to be not at all stable.

In *Bouteloua gracilis,* Fults (1942) found a range of biotypes in plants collected from different geographical regions. The 2n chromosome counts in these plants were 21, 28, 35, 42 and in plants from two sources 61 and 77. With one exception there was no correlation between plant type and chromosome number. A similar situation including the occurrence of aneuploids was found in *Bouteloua curtipendula.*

Stebbins and Love (1941) examined the chromosome complements of twenty-two different specimens of *Bromus carinatus* which were widely divergent in morphological characters. Despite the variations, the 2n number in all cases was found to be 56.

At present the data from the Rhode Island specimens do not warrant any conclusions as to the reasons for the occurrence of frequent aneuploids or whether all of these plants should be considered *A. tenuis* Sibth., *A. alba* L. or hybrids between the two species.

SUMMARY

Widely divergent morphological types were found within single progenies of *Agrostis* collected in Rhode Island. These differences were greater than is usually found within a species, and individual plants could be identified as *A. tenuis* Sibth., *A. alba* L. or intermediates between these species. Chromosome counts of these plants ranged from 28 to 42 with no correlation between morphological type and chromosome number. At present no explanation is offered as to the reason for the occurrence of these aneuploid chromosome numbers or for the morphological variations.

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STRUCTURAL COMPOSITION OF THE SCLEREIDS IN THE INTEGUMENT OF PISUM SATIVUM L. [1]

R. M. Reeve

PROBLEMS OF texture constitute a major field of technological investigations in the commercial processing of vegetables as food. The causes of texture change in the seed coats of beans and peas intended for freezing or canning not only involve biological variables but also the effects of harvesting, handling, and processing treatments. Food research has been confined mainly to these latter environmental effects on the qualities of the processed products. Existing information on cell-wall composition and other histological factors of texture has been either incomplete or unreliable, particularly with respect to the chemical nature of the secondary wall structure in the epidermal cells of the seed coat. The histochemical studies reported here were undertaken in order to clarify confusing information and to establish facts on the compositional nature of the tissues responsible for seed-coat texture. The results of these studies are of direct interest to plant anatomy and should provide significant and useful information to applied research in commercial food preservation.

ANATOMY OF THE INTEGUMENT.—*Structure of the epidermis and hypodermis.*—Many detailed accounts have been written about the anatomy of the ovule and integument in leguminous species (Pammel, 1899; Netolitzsky, 1926; Hamly, 1932–1935; Zimmerman, 1936; and others). Therefore, it should be necessary to present only a general description of the cellular organization (fig. 1, 2, 3). In beans and peas the epidermis is highly specialized at maturity into elongated cells with thickened walls and possesses a well-defined cuticle. These cells have been variously designated as Malpighian cells, prism cells, palisade cells, and *macrosclereids*. Underlying them is a hypodermal layer composed of specialized cells which have been called "I" cells, or "hourglass" cells. The term "Tragerzellen," used by Zimmerman (1936) and previous investigators, is well suited because of their obvious resemblance to pillars supporting the epidermis (fig. 3). The terms *macrosclereids* and *osteosclereids* are used here for the cells of the epidermis and hypodermis, respectively, because their histological classification is considered more desirable than descriptive reference to their appearance (see Foster, 1942, on tissue classification and chapter on sclereids where Tschirch is cited).

The macrosclereids are further characterized at maturity by fluted, twisted wall thickenings which are most pronounced in the apical portions of the cells. These thickenings undergo considerable increase during growth in the harvest season (fig. 4 to 7 and 10 A–B). In the basal third of the cell they are less pronounced, except at the extreme basal end, and the secondary wall surrounding the lumen is more or less uniform in outline (fig. 6, 7 and 10 A–B). At the extreme basal end of the macrosclereid the lumen is constricted further by a uniform secondary wall thickening which may become more or less pronounced as the integument of the pea seed matures (fig. 3).

The much discussed "light line" of this tissue layer may be observed always near the apical ends of the cells, in close proximity to the termination of the lumen and where the fluted thickenings occupy nearly all of the cell cavity and converge to form the end wall. The lumen is extended between the thickenings as minute, finger-like slits which some of the earlier literature describes as "pore canals" (fig. 4, 5). They have no relationship to the primary and secondary wall pits of present-day plant anatomy and are only the result of the crowding or filling of the cell cavity by the specialized wall thickenings.

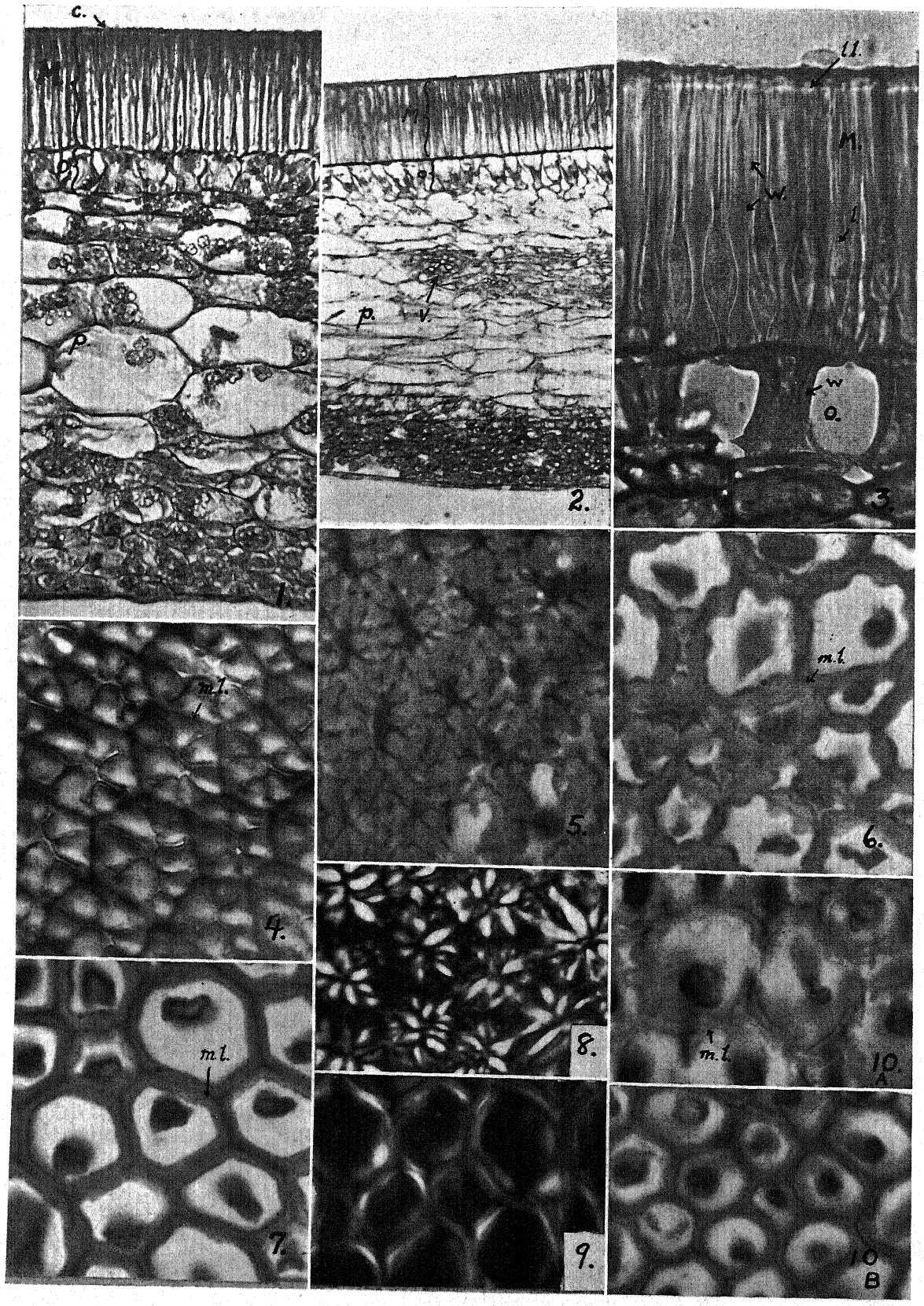
The osteosclereids also possess thickened longitudinal walls, the secondary wall development being most evident along the constricting curvatures of the central portion of the cell. Near the hilum region, and to a lesser extent elsewhere in the integument of the pea, the thickenings may be somewhat fluted and slightly spiraled (fig. 16). In garden varieties of peas and lima beans these cells are somewhat more uniform in shape and structure than in many other leguminous species, in which they display considerable variety in form (Pammel, 1899; Tschirch and Oesterle, 1893; Netolitzsky, 1926; Hamly, 1932; Zimmerman, 1936; and others).

Underlying the osteosclereids is a loosely organized parenchyma (fig. 1, 2). In many genera there are further tissue specializations in the integument, and the endosperm may be well defined with aleurone cells (Pammel, 1899, and Zimmerman, 1936). In the common garden varieties of beans and peas these other tissues are ill defined or lacking when the ovule is approaching maturity.

The macrosclereids at the hilum region form a double layer under which the osteosclereids are modified into a special zone surrounding a pocket of reticulate, lignified sclerenchyma. Approaching this region, the osteosclereids may be somewhat more elongate than elsewhere in the integument. Along the top of the seed, opposite the hilum and at the strophiole region, the macrosclereids become elongated, frequently two or three times their normal length over most of the seed coat (Zimmerman, 1936). Otherwise the structural characteristics and composition of these two layers are more or less uniform in the integuments of beans and peas.

Significance of specialization.—Functioning of the integument in germination of leguminous seeds has

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been a subject for research over a number of years. Hardness of the seed coat, the strophiole and micropyle structures and various tissue specializations in relation to water retention, water absorption, longevity, etc., are among the many aspects which have been investigated in plant histology, physiology and by chemical analyses.

The earlier plant histologists were handicapped by lack of specific reagents for detection and differentiation of cell wall substances *in situ*. Most of the tests made upon cell walls were confined to the swelling action of acids and various solutions, solubility, and dyes which differentiate structure but are not specific for chemical composition. Exceptions of course were the classical tests for cellulose and lignin (the action of iodine and phloroglucinol, respectively) but even these provided only partial evidence. Thus the tabularized results of microchemical tests made on the integuments of various members of the Leguminosae by Zimmerman (1936), and particularly of the earlier publications by Pammel (1899), Tschirch and Oesterle (1893–1897), Marlière (1897), Harz (1885) and others, describe reactions which primarily concern cellulose without revealing any information as to its orientation or the pentosans which may be associated with it.

The presence of pentosans and "hemicelluloses" in seed coats has been quite commonly assumed, but little histological research has been accomplished to demonstrate their relationship to structure and function. Norman (1937) and Ott (1943) present excellent reviewing chapters on the subject of cellulose and its associated pentosans, and Norman discusses the metabolism of cell wall substances. Van Wisselingh (1925) has considered the composition of plant cell walls in various groups of the plant kingdom in some detail, and Hawley (1932) has presented a classification of the "hemicelluloses."

Martin and Watt (1944), in studies on *Melilotus alba* and *M. officinalis*, came to the general conclusion that the cuticle, the "light line" and the thickened walls of the macrosclereids form an almost continuous barrier to water absorption. However, they did not attempt any determinations of cell wall composition. Ott and Ball (1943) analyzed seed coats of the common bean and found a relationship between chemical composition and water retention. They also reported, on a per cent dry-weight basis, averages of about 19 per cent polyuronides and 21 per cent "true pentosans." Their results are in general agreement with other published data.

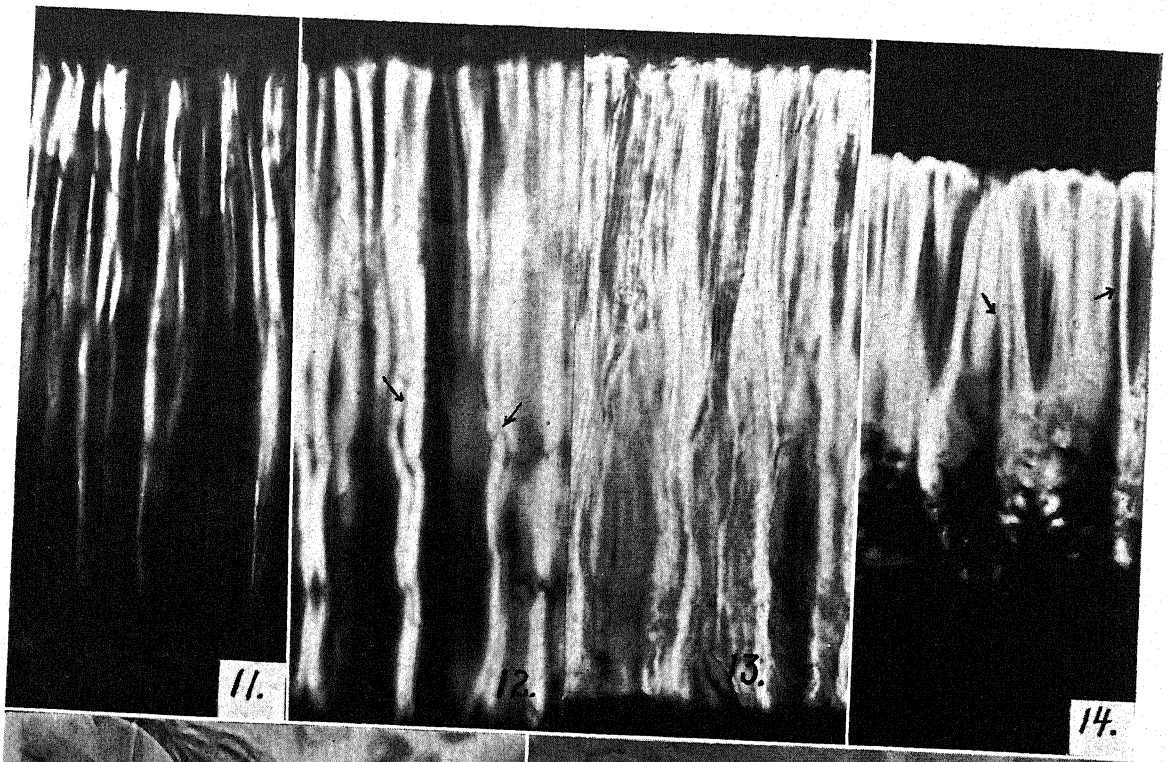
In recent years sufficient knowledge of the structure and compositional characteristics of cell walls has been accumulated so that the association of lignins, "hemicelluloses" and pentosans with the cellulosic matrix can be investigated by microscopic methods. General, and in many cases specific, agreement between chemical data and histological interpretation is found in the researches of Bailey and his coworkers (1935, 1937, 1940, 1942), Berkley (1939), Bennett (1940), Buston (1935), and others.

HISTOCHEMICAL TESTS.—Reagents which yield characteristically colored reaction products with the carbohydrate constituents found in plant cell walls should prove of considerable value in plant anatomy. The results can be verified by simple chemical analyses, if the quantity of the material permits, or by testing known carbohydrates.

The accompanying table summarizes the results obtained with several tests which have been adapted to microscopic methods. Reliability of the reagents used is indicated first by the fact that they have been applied in carbohydrate chemistry as qualitative indicators, and second by the use of reaction products as bases for quantitative methods of pentose determinations (Morrow and Sandstrom, 1935; Dehn, Jackson, and Ballard, 1932). Their dependability in histological application, which involves a structural complex of mixed sugars in the cell wall, was verified by tests on samples of xylan from barley straw, crude and refined pectins, and native and refined cotton. It is necessary, however, to consider various aspects of the results of these tests on the tissues of the integuments in more detail.

In general, preparations made from dried and finely ground tissues (20-mesh screen) proved to be more adaptable to these tests than either fresh sections or preparations made from paraffin-embedded material. When the results of a test produced only slight coloration, sections of young tissues sometimes proved to be too thin to show more than a faint tint when observed microscopically. The hardness of the macrosclereids produced further difficulties in obtaining suitable sections. The ground tissues would macerate sufficiently to permit observations on intact individual cells.

Fig. 1–10.—Fig. 1. Longi-section through integument of young pea ovule. "C" = cuticle, "M" = macrosclereids, "O" = osteosclereids, "p" = parenchyma. Note abundance of starch in young integument. Section cut from "Alaska," a small-seeded variety. ×240.—Fig. 2. Integument of young lima bean ovule, showing vascular tissue (V) in the parenchyma (p). ×140.—Fig. 3. Longi-section of macro- and osteosclereids in the mature integument of *P. sativum* (about sieve size 5). "ll" = light line, "l" = lumen, "M" = macrosclereids, "O" = osteosclereids, "W" = thickened walls. Commercial sieve sizes, expressed in fractiors of an inch, are: 1 = 9/32, 2 = 10/32, 3 = 11/32, 4 = 12/32, 5 = 13/32, 6 = 14/32, 7 = 15/32.—Fig. 4. Transverse section of mature macrosclereids of *P. sativum* (about sieve size 5), just beneath the apical end, or near the "light line." "M.l." = middle lamella. ×1,200.—Fig. 5. Approximately the same level as figure 4, but younger stage (about sieve size 2 or 3). ×1,200.—Fig. 6. Transverse section through upper part of basal half of the macrosclereid layer (about sieve size 5). ×1,200.—Fig. 7. Through lower part of the basal half of macrosclereid layer (about sieve size 5). ×1,200.—Fig. 8. Approximately same level as in figure 4, viewed with crossed nicols (see text). ×1,200.—Fig. 9. About same level as figure 7, viewed with crossed nicols. ×1,200.—Fig. 10a. Same level as figure 6, but a younger stage (sieve size 2 or 3). ×1,200.—Fig. 10b. Same level as figure 7, but a younger stage (sieve size 2 or 3). ×1,200.


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Phloroglucinol-hydrochloric acid test.—Zimmerman's (1936) conclusions were based primarily upon results which show differential solubilities and rather generalized staining differentiation of cell wall regions which would not necessarily characterize any pentosan-cellulosic complex. His use of the phloroglucinol reagent did not result in a positive "hemicellulose" or pentosan reaction, either in the secondary wall thickenings of the macrosclereids or in the osteosclereids, because the preparations were not heated. Therefore, he regarded the upper portions of the walls of the macrosclereids as being composed of nearly pure cellulose. Hamly (1932) arrived at essentially the same conclusions for *Melilotus alba,* but suggested that some "hemicellulose" might be present in the secondary walls of the macrosclereids. Hamly's methods likewise would not differentiate the pentosan components from cellulose, and his suggestion is based upon results obtained with the *hydrocellulose* reaction. This test involves treatment with dilute (60 to 70 per cent) sulfuric acid, followed by staining with I–KI. Certain "hemicellulosic" materials as well as cellulose will yield an "amyloid" blue color (Rawlins, 1933).

It should be noted, as indicated in the table, that the phloroglucinol-hydrochloric acid test was applied in four modifications. The more common histological use of this test involves treatment of the tissues with about a one per cent solution of phloroglucinol in alcohol, followed by the addition of a few drops of hydrochloric acid, either concentrated or diluted to 50 per cent. According to Johansen (1940) this test is specific for lignin when used cold or unheated, but upon heating the pentosans of xylose and arabinose yield a color reaction very similar to that for lignins. Stark and Mahoney (1942) used it for lignin and pentosans in bean pods. The other phloroglucinol reagent, a saturated solution in 50 per cent hydrochloric acid, is common to carbohydrate chemistry (Morrow and Sandstrom, 1935).

Nearly identical color reactions were obtained with these reagents, in their corresponding treatments. The acid solution of phloroglucinol was perhaps more sensitive and reliable as a pentosan indicator than the alcohol solution. Particular care was taken to avoid overheating the preparations because the characteristic color reactions which distinguish xylose and arabinose pentosans (red or pink, grading into lavender) from those of the methyl pentoses (yellow orange to dark orange) are those which occur before the formation of dark furfural precipitates. The preparations were heated intermittently over a flame for about five minutes so that a high temperature could be reached without violent boiling and disruption of the tissues. Placing the slides either over flowing steam or on a hot plate was satisfactory but required a longer treatment, and the beginning of color development was not easily detected in time to make microscopic observations.

Bial's orcinol test.—This method also is used in carbohydrate chemistry for differentiation between pentoses and methyl pentoses. It proved to be very reliable as a histochemical test in these studies. The reagent consists of a dilute solution of orcinol in concentrated hydrochloric acid (1:500) to which are added a few drops of 10 per cent aqueous ferric chloride solution (about ten drops per 100 cc.). Several drops of the reagent were added to the tissues and heat was applied with the same caution used in the previous tests.

It was found that different concentrations of acid and orcinol and varying amounts of the ferric chloride solution produced nearly the same results, but the test appeared to be most sensitive for histochemical purposes when the reagent was prepared as described. The pentoses, xylose and arabinose yield a green color which changes to blue-green and bright blue before any turbidity develops. Methyl pentoses, on the other hand, produce red or orange color reactions (Bial, 1902; Morrow and Sandstrom, 1935; van der Haar, 1920).

When Bial's reagent is applied to the pea integument and carefully heated, a differentiation between the pentosans of the secondary wall thickenings and the polyuronides of the middle lamellae of the macrosclereids and osteosclereids is obtained. A characteristic light but bright green color develops in the wall thickenings and changes to bright blue green and blue, with additional heating, before ultimate hydrolysis occurs and the color diffuses into the surrounding medium and becomes darkly turbid. The middle lamellae of all the tissues become noticeably red in contrast to the green and blue shades. Only an extremely faint color formation occurs in the walls of the parenchyma, except for portions in close association with tissues yielding the characteristic

Fig. 11–18.—Fig. 11–14.—Longi-sections (5 μ) through macrosclereids of different ages viewed between crossed nicols. ×1,200.—Fig. 11. Stage of development at sieve size 2.—Fig. 12. Sieve size 3, or younger of sieve size 4 (arrows—middle-lamellae).—Fig. 13. Sieve size 5.—Fig. 14. Sieve size 7, nearly ripened seed (arrows—middle lamellae). Increased development of the fluted wall thickenings in this series is indicated by increased anisotropy since the sections are of the same thickness. In addition, this series represents comparable areas of the integument because each photomicrograph shows a region at the greatest horizontal diameter of median sections cut perpendicular to the long axis of the hilum. The same variety (Alderman) is shown in each, and harvest was made from plants growing under identical environments. Note that "ripening" involves not only a pronounced secondary wall development, but also a shrinkage in length of the macrosclereids (compare 13 and 14).—Fig. 15. Macrosclereids swollen with 50 per cent sulfuric acid and photographed with ultraviolet illumination to show fibrillar orientation. The large macrosclereid is one from the strophiolar region. ×800.—Fig. 16. Transverse section through the osteosclereid layer showing the pronounced secondary wall thickenings in the constricted, equatorial region. ×1,200.—Fig. 17. Macrosclereid swollen with sulfuric acid and photographed with ultraviolet illumination to show the fibrillar orientation. ×3,000.—Fig. 18. Upper half of a swollen macrosclereid. ×2,500.

TABLE I. *Summary of histochemical tests.*

Tests and reagents	Secondary walls of macrosclereids: Apical end	Secondary walls of macrosclereids: Basal end	Middle lamellae	Secondary walls of osteosclereids	Lignified tissues
Alcohol solution of phloroglucinol + HCl (test for lignin)	No reaction	No reaction	No immediate reaction	No reaction	Rose-red to lavender
Alcohol solution of phloroglucinol + HCl, heated (test for pentosans)	Lavender-pink	Light lavender-pink; red tint bordering middle lamellae	Deep red-orange (see text)	Lavender-pink	Color destroyed by heat
Hydrochloric acid solution of phloroglucinol (test for lignin)	No reaction	No reaction	No immediate reaction	No reaction	Rose-red to deep red-lavender
Hydrochloric acid solution of phloroglucinol, heated (test for pentosans)	Bright lavender-pink	Lavender-pink; red tint bordering the middle lamellae	Deep red-orange (see text)	Lavender-pink	Color destroyed by heat
Bial's orcinal test (for lignin)	No reaction	No reaction	Very faint pink	No reaction	Bright rose-violet
Bial's orcinol test, heated (test for pentosans)	Green, changing to bright blue-green, blue or turquoise	Same as apical end, but lavender next to middle lamellae	Deep red (see text)	Green, changing to blue-green and light blue	Rose-violet, changing to green; eventually turbid brown
Hydrochloric acid solution of orcinol (test for lignin)	No reaction	No reaction	Red tint	No reaction	Rose
Hydrochloric acid solution of orcinol, heated (test for pentosans)	Green to blue-green	Green to blue-green	Orange-red (see text)	Light green to blue-green	Rose, changing to green; eventually turbid brown
Methylene blue chloride (for structural differentiation) 1:10,000 dilution	Light green, grading at middle of cell to blue-green	Blue-green to blue and violet	Violet	Green to blue-green	Faint green

pentosan reaction. Here the green color development is slightly more pronounced but less apparent than in the macrosclereids and osteosclereids.

In the lower portion of the macrosclereid layer, where the middle lamellae appear to be more pronounced than in the upper portion, there appears to be some slight pink or red color development into the secondary wall. This color may indicate the presence of some methyl pentosans, as found in pectins, or it may be merely a diffusion of the colored compound from the middle lamellae. It seems probable that pectic materials related to those of the middle lamellae can be found in the secondary wall; thus the former interpretation is logical. Also, in fully matured ovules, the middle lamellae of the macrosclereids give some indication of encrusting pentosan materials. This indication is most evident when small portions of the ground integument are pretreated with dilute ammonium oxalate (0.5 per cent) or dilute hydrochloric acid (0.1–1.0 per cent) to remove certain pectic fractions and then washed thoroughly with distilled water before the Bial's reagent is applied. With such treatment the middle lamellae may show a distinct tendency toward development of the green color reaction, but it is somewhat obscured by the presence of methyl pentosans, or substances which yield the red reaction, remaining in the intercellular substance. However, one might expect deposition of encrusting pentosans during maturation of those tissues (Bailey, 1940; Bailey and Kerr, 1935; and Norman, 1937).

With this test the wall thickenings in the region of the "light line" take a more pronounced color than they do along the lateral areas of the cells. Since it is at this level that the fluted thickenings converge over the end of the cell and form the "end" wall, the slightly increased color can be seen to be related to the increased amount of wall substance, *i.e.*, a thicker layer of secondary wall containing pentosans. Sections cut tangentially to the surface of the integument and tested with Bial's reagent do not indicate any chemical differentiation of the "light line" from the rest of the secondary wall in the upper regions of the cells. This observation was verified by similar observations with the phloroglucinol tests previously described.

Differentiation between lignin and pentosans.—Bial's test also may be used to distinguish between lignin and skeletal "hemicelluloses" or pentosans. When applied to both sections of pod and ovule, without heating, the lignified walls of xylem, the reticulate, lignified thickenings of the sclerenchy-

matous pocket under the hilum, and the "parchment" layer of the pod respond with a lavender to red violet color characteristic of the ordinary orcinol-hydrochloric acid reagent which may be substituted for the phloroglucinol test for lignin. However, no color is developed in the secondary wall thickenings of the macrosclereids and osteosclereids.

When heat is applied, Bial's test produces a deep green in the lignified walls, and in the pentosan-containing walls of the macrosclereids and osteosclereids it produces a more delicate green changing to blue-green as previously described. The cell contents (nuclei, starch grains and cytoplasmic granules) of the parenchyma in the integument and the cotyledons may also develop a green color, and sometimes some protoplasmic components develop a red tint. One would expect such reactions with the presence of different pentoses in the cell contents. However, these reactions do not interfere with the specificity of the reagent for cell wall substances that are further defined by structural characteristics, since it is easy to obtain differentiation of pentosans, methyl pentosans and lignin by their color reactions in sections of tissue devoid of starch and with little or no cell content. Moreover, the more soluble sugars of the cell contents may be removed by treatments which have little or no effect upon the more resistant wall structure. Thus it can be verified that the characteristic cell wall reactions are not caused by absorption of colored compounds formed in the cell contents.

When orcinol and hydrochloric acid are used without the ferric chloride, only slight application of heat destroys the typical lignin reaction. All cell walls take on a faint green tint, and with additional heating the green becomes more pronounced, particularly in the thickenings of the macrosclereids. However, the test is less sensitive than the regular Bial's test and appears to have less capacity for differentiating between pentosans and methyl pentosans.

Further distinction of the pentosan wall structure of the macrosclereids from lignified tissues is shown by the negative results obtained when the Maule test for lignin is applied to preparations of the pea integument. In this test sections and ground samples were treated 15 minutes or more with 1 per cent potassium permanganate; then they were washed thoroughly with distilled water. Next the preparations were treated with 2 per cent hydrochloric acid for 5 minutes and again washed. Two or three drops of 5 per cent sodium bicarbonate solution were then added to the tissues. No red or brown coloration could be distinguished in any cell wall regions of the integument other than in those tissues already described as lignified and showing red or violet color with the cold phloroglucinol-hydrochloric acid test for lignin. In the Maule test manganese dioxide and hydrochloric acid react with lignin to form a lignin chloride which forms a red compound with the addition of the bicarbonate solution (Rawlins, 1933).

Aniline-hydrochloride test.—When preparations mounted in dilute or concentrated hydrochloric acid were heated carefully for one to five minutes and then treated with aniline hydrochloride, a bright red color generally developed in the solution and usually in close proximity to the macrosclereids and osteosclereids. Frequently the thickened walls of these cells developed a red color, which appeared to vary with the length of time the preparation had been heated and with acid concentration.

Various modifications were attempted, such as treating the tissues with aniline hydrochloride before treatment with acid. These different manipulations did not result in improvement over the procedure which more nearly parallels the chemical use of this test. Although positive results indicative of cell wall pentosans were obtained and were in agreement with the phloroglucinol and Bial's tests, the test is neither convenient to apply nor is it as adaptable to histological materials as those previously described.

Other treatments and analytical results.—Following much the same procedures as described by Kerr and Bailey (1934), both sectioned and ground samples of the seed coats were variously treated with oxalic acid, ammonium oxalate, and dilute hydrochloric acid (0.1 per cent) to remove the pectic constituents. With ordinary stains such as ruthenium red and methylene blue chloride (in dilutions of 1:5,000 and 1:10,000), these treated preparations showed a marked decrease in the staining of the middle lamellae. However, the treated preparations still showed very distinct color reactions in the secondary wall thickenings of the macrosclereids and osteosclereids when tested with the phloroglucinol and Bial's reagents. Also, the middle lamellae still gave characteristic reactions to both tests as previously described, indicating the presence of resistant materials.

The only tissues in which this pectin extraction appeared to have a pronounced effect were from the integuments of immature peas, in which little or no secondary wall thickening of the macrosclereids was evident. The tissues, of course, macerated more readily than the older integuments and it was evident that they contained either little "encrusting hemicellulose" or less resistant material in the middle lamellae.

In addition to treatment of tissues on slides for microscopic observation, larger samples of dried, ground and nearly mature seed coats were treated in a reflux extractor for several hours with 4 per cent sulfuric acid. Pectins and hemicelluloses are removed from most plant tissues by this treatment. The crude extract amounted to about 15 per cent of the original dry weight of sample. The residue, however, showed little evidence of tissue maceration and still gave characteristic cell wall pentosan reactions, as previously described, when tested with the phloroglucinol and Bial's reagent. If anything, the preparations made from the extracted residues showed the distinction between the cell wall pentosan reac-

tions and the materials of the middle lamellae more readily than did untreated samples. Possibly the extraction had resulted in some partial hydrolysis of the more resistant form of pentosans in the secondary walls so that further hydrolysis and color formation were facilitated; or removal of other more soluble forms of sugars may have rendered the tests more sensitive.

Very similar results, both as to amount of crude extract and the response of the residue to histochemical tests, were obtained with samples alternately extracted with 4 per cent cold and hot potassium hydroxide for several hours. Each of these methods should result in removal of at least some of the hemicellulosic fractions from plant tissues, but it is obvious from the results of tests made upon the residues that the form of pentosan existing in the secondary wall thickenings of the macrosclereids and osteosclereids is resistant to hydrolysis with weak reagents.

Another larger lot of dried, ground samples was extracted with hot dilute sulfuric acid for a longer period and the filtrate treated to obtain pentose sugars according to methods described by Morrow and Sandstrom (1935). The amount of material available did not permit separation and purification procedures; thus methods of identification in mixture were used (van der Haar, 1920). Two and possibly three types of crystalline osazones were obtained and tentative identification was based mainly upon microscopic appearance as illustrated by Hassid and McCready (1942), Morrow and Sandstrom (1935) and others. They also were compared with osazones prepared from known sugars. One form was unmistakably characteristic of arabinosazone; the other two were similar in appearance to galactosazone and the phenylhydrazone of galacturonate. Arabinose might be expected to occur as araban structurally associated with cellulose in the secondary walls, and it also could be associated with galactose and uronic acids as a constituent of the middle lamellae.

It was not possible to obtain the osazone of xylose, but this sugar was identified in the mixture, possibly in its xylonic acid form, by Bertrand's reaction. This test depends upon an interaction of xylonic acid and hydrobromic acid with cadmium carbonate to form, on concentration, characteristic boat-shaped crystals of the double salt cadmium bromide xylonate. Other sugars yield different and less characteristic crystal forms. Apparently the xylose hydrolized from the tissues during extraction had been converted to its acid form.

While no further attempts were made toward chemical identification, these results are considered reliable for histological purposes. They are in agreement with the histochemical tests that have been described and with chemical information on cell wall composition. The pentosans of xylose and arabinose are fairly common constituents of seed coats, and although xylan is not known to exist free in plant tissues as are some other pentosans, it is believed to be incorporated into the cellulose chain in some manner such as has been suggested for the Cross and Bevins "cellulosan" (see Norman, 1937, for a discussion of the hemicellulosic pentosans). Other investigators have reported xylose and arabinose as components of the pod and seed coat tissues of other leguminous species (Norman, 1937, and Ott, 1943). To my knowledge histological application of pentosan tests designed to demonstrate the location of these materials (other than the phloroglucinol test) have not been previously reported in literature on seed plants.

SUBMICROSCOPIC STRUCTURE.—*As revealed by staining.*—The differential staining of the wall regions and middle lamellae by methylene blue chloride (1:10,000) leads to several interesting considerations of cell wall structure. This dye is *metachromatic.* Research by Czaja (1930) indicates that its differential staining capacity and that of related dyes, when applied to such structures as plant cell walls, may be accounted for by differences in size of dye "particles." Thus material that limits or prevents absorption of particles above a certain size will be stained more lightly, or even differently, from substances that allow all and larger-sized particles to penetrate. This theory of staining as applied to protoplasmic contents may be open to question, but it appears to be characteristic for cell wall structure.

Griffioen (1938), in research on the origin of lignin in woods, found that young elements with little or no lignin stained a characteristic blue, and, as lignification of the secondary walls increased, less dye was absorbed until in many of the older elements no staining could be observed. This principle appears to operate only when extreme dilutions of the dye are used.

In sections cut from older integuments of peas it can be seen that the walls and middle lamellae of the macrosclereids show a very similar staining with methylene blue chloride. It is interesting to note that the regions of the secondary walls of the macrosclereids and osteosclereids that stain a light green are those in which resistance to treatment is apparent and in which the characteristic pentosan results were obtained with phloroglucinol and with Bial's reagent.

If we assume the point of view on "cellulosan" structure discussed by Norman (1937), it is possible to interpret a relationship of this restricted staining to the micellae of the pentosan-cellulose chains. Likewise, in the case of "encrusting" pentosans or "hemicelluloses," the intermicellar spaces of the cellulosic matrix would be reduced in size and would limit the amount of dye absorbed.

Additional evidence on submicroscopic structure of the walls of macrosclereids is found in the fact that with increased age these secondary walls stain more lightly. In the integument of ripened ovules the staining capacity of the fluted thickenings is

visibly a lighter green with less blue than in stages with thickenings only well defined (see fig. 11 to 14 for development of thickenings of different ages). The difference between the basal part and the apical part of the cell may be interpreted similarly (see table).

Cellulose orientation.—Bailey (1940) and Bailey and Kerr (1935) have shown evidence that lignin occurs as an amorphous deposition in the interstices between the cellulose fibrils of the secondary wall. These fibrils are not considered as discrete entities and should not be confused with "chains." They may be composed of chains of cellulose molecules, but the term is used for convenience as descriptive of the physical appearance of treated tissues. Bailey and Vestal (1937) have demonstrated the orientation of cellulose fibrils in the secondary walls of tracheary cells by treatment with iodine and dilute sulfuric acid. With this technique iodine crystals are formed in the elongated interstices between the cellulose fibrils. The close correlation of evidence obtained by this method with that of optical properties (polarized light) and x-ray diffraction patterns is discussed by Bailey and Berkley (1942).

When the iodine-sulfuric acid method was applied to the macrosclereids of peas, results very similar to those just described were observed. However, a much finer network of cellulose fibrils occurs in these cells than is found in tracheary cells. Consequently, the finely dispersed iodine precipitations sometimes hindered rather than facilitated interpretation of the fibrillar orientation. Likewise, the presence of pentosans, which will yield a "hydrocellulose" reaction readily when treated with strong acid and iodine, produced further complications of observation, and more dilute solutions of acid did not result in sufficient swelling of the walls to bring out the fibrils sufficiently to be resolved by the microscope.

When concentrations of 50 to 60 per cent sulfuric acid were used without iodine, it could be seen that two opposing helicles of cellulose fibrils were present in the secondary walls of both the macrosclereids and the osteosclereids. These helicles have a pitch of about 30° or less (fig. 15, 17). The preparations were photomicrographed with ultraviolet illumination to increase resolving power and permit greater magnification.

The upper portion of the macrosclereid frequently is greatly expanded by this acid treatment and the fibrillar structure is somewhat obscured. There appears to be an intertwining of the fibrils into the fluted thickening, but its exact nature could not be determined (fig. 15, 17, 18). Apparently the severe swelling is caused by the more pronounced hydrolysis of the pentosans present in these thickenings. The "hydrocellulose" reaction previously mentioned is usually obtained with the hydrolysis of the cellulose when either concentrated or 70 per cent sulfuric acid is used on cellulose walls. However, in these wall regions where expansion is greatest with only 50 per cent acid, a widely spread gelatinous mass stains intensely blue with iodine. With chlor-zinc-iodide the reaction is similar, but the swelling is less pronounced. Zimmerman (1936) noted this difference in swelling between the primary and secondary walls of the macrosclereids of other legumes, but he did not associate it with pentosan content.

Macrosclereids of the strophiolar region show a similar cellulosic orientation, but their swelling is somewhat more uniform and there does not appear to be as pronounced flaring in the apical portions of the cells (fig. 15). This difference appears to be largely structural, since the fluted wall thickenings which traverse most of the lengths of these longer cells yield the same results with the pentosan test as do the shorter cells.

No pronounced distinctions in fibrillar structure could be distinguished between primary and secondary wall layers other than those already described in reference to the fluted thickenings. Before any thickenings have developed, it is apparent that the thin walls of the young macrosclereids are uniform in thickness and may be described as "primary" walls. Microchemical tests show the presence of some pectic materials, but the formation of the pentosan-cellulosic complex is characteristic of the walls when the fluted thickenings have begun to form and the macrosclereids develop their specialized structure.

Optical properties.—*Polarization characteristics.*—When 5 μ sections, cut longitudinally through the macrosclereids, are examined between crossed nicols and compared with transverse sections, it is apparent that the cellulosic crystallite orientation parallels the lengths of the fibrils (fig. 9 and 11 to 14). Because of variations in thickness of wall materials and the angular direction of the fibrils, there is no over-all sharp extinction position for any individual macrosclereid. Figures 13 and 14 show gray to black streaks where it is apparent that areas within the wall thickenings are near the position of extinction while other regions of the secondary walls are in position of greatest brightness or nearly so. Longitudinal sections show a strong birefringence of the secondary wall thickenings (fig. 11 to 14), and transverse sections are only slightly birefringent except for sections cut through the apices of the cells (compare fig. 8, 9).

Further verification of these optical properties was gained by more detailed observations on macrosclereids of different stages of development. When 5 μ longitudinal sections of older macrosclereids are rotated between crossed nicols, the lateral wall portions of the fluted thickenings show blue, orange-red, and purple shades of polarization colors. These colors may be observed when the cells are in position of greatest brightness, but even in position of least brightness the colors are still evident. Sections of the same thickness, but of less mature macro-

sclereids from unripened seeds, show a sharper extinction and a decreased range of polarization colors.

The differences between younger and older cells in sharpness of extinction when examined between crossed nicols indicate an interesting relationship to several conditions of cell wall development. During maturation of the macrosclereids there not only is development of additional wall substances in the fluted thickenings, but it is apparent that these cells become shorter as the seed "ripens" (fig. 13, 14). This unique characteristic of shrinkage may be related to the effects of natural moisture loss upon the wall structure. Zimmerman (1936) has reported an increase of about 11 per cent in length for the macrosclereids of ripe *Phaseolus vulgaris* seed coats when soaked. The author has found no reports of the sort of shrinkage just described. Additional information from x-rays and histogenetic studies in progress may provide a better understanding of these unusual structural characteristics and their behavior during maturation.

Interpretation of the polarization colors which have been described necessitates the use of a compensator (fig. 19a–b). When a first-order red plate is used and the macrosclereids are oriented with their long axes parallel to the slow axis of the plate, the thinner portions of the walls (mainly the basal half of the cells originally first-order gray) appear blue. In this position, the fluted thickenings show a range of colors of higher order than the original. When the cells are in position so that their long axes are at right angles to the slow axis of the plate, retardations are subtracted. In this position the fluted thickenings are predominately yellow or yellow-orange, with some slight gray streaks.

Since the polarization colors of the older macrosclereids show considerable range, they may be best interpreted by the use of a quartz wedge compensator (see Chamot and Mason, 1938, for details on retardation). When the sections are placed so that the long axes of the cells are at 45° to the vibration axis of the analyzer and perpendicular to the slow axis of the quartz wedge, the nature of the polarization colors may be determined. The thickenings of the walls of the older cells show a range of colors from first-order gray up to and including the bordering purple of the second order. By compensation it can be verified that the crystallite orientation of the secondary walls and their fluted thickenings is more or less lengthwise of the cell except for the apical end where the thickenings are joined to form an end wall, and where the crystallite orientation tends to be at right angles to the long axes of the cells (compare *a* and *b* in fig. 19). This is based upon the assumption that the slow ray within the crystallite is longitudinal.[2] When the compensator is used and retardation is additive for the radial, longitudinal walls of the macrosclereid, compensation of the color in the tip portions of the fluted thickenings is obtained. The same condition of optical characteristics may be observed at the basal end of the cell wall. Here the thickening is uniform, but the crystallite orientation changes direction over the end wall.

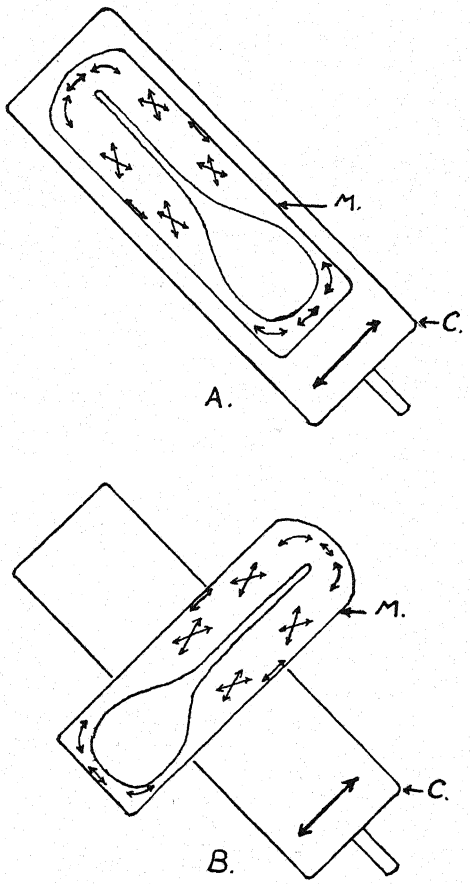


Fig. 19. Idealized diagram of the positions of the macrosclereid (M) when showing greatest brightness (or nearly so) when superimposed upon the position of the compensator (C) and examined between crossed nicols. Arrow on compensator indicates the direction of its slower component. Arrows on the macrosclereid indicate approximate direction of cellulosic fibrils and their crystallite orientation. A—position when lateral walls show compensation. B—position when end walls show compensation.

Careful observation of sections of the same thickness (5 μ) of older and younger macrosclereids show that differences in thickness of wall substance, due to both increased wall material and overlapping portions of fluted thickenings, and possibly an increase in the degree of crystallinity, are responsible for the increased complexity of the polarization colors in the older macrosclereids. The position of the cells when showing brightest anisotropy and the longitudinal direction of the slow ray provide reliable indication that the cellulosic crystallites are oriented lengthwise to the macrosclereids.

[2] The validity of this assumption has been established by x-ray diffraction patterns which are similar to those for ramie and show that the macrosclereids of peas have a highly oriented crystallite structure, and that, in the radial walls, the short ray is parallel to the longitudinal axes of the fibrils.

Essentially the same results were obtained with acid-swollen cells observed with polarized light and interpreted with the compensators. In fact, it could be observed that considerable swelling (fig. 17, 18) resulted before there was any pronounced decrease in birefringence. With the upper wall regions greatly expanded (fig. 18) there was a marked decrease, but the vibration plane of the slow ray remained parallel to the long axis of the cell. The retention of birefringence in these acid-swollen preparations suggests that the crystallites of cellulose may be much more compact and resistant than one might expect. It is suggestive of the "cellulosan" structure (Norman, 1937).

Bailey and Berkley (1942) have described in detail various aspects of fibrillar orientation, optical properties, and x-ray diffraction patterns of the secondary walls in wood cells. They have pointed out that where the cellulosic fibrils of an outer layer are oriented at right angles to longitudinally oriented fibrils of an inner layer, there may be areas of constriction when the walls are swollen with acid. This characteristic phenomenon, well established in other research and discussed by Bailey (1940), was investigated carefully in an attempt to account for the flaring of the upper wall regions in the macrosclereids of peas when swollen with acid. No evidence of this sort of difference in fibrillar orientation could be found in dry ground samples or in sections variously treated to swell the walls.

It seems reasonable, therefore, that the flaring of these cells when treated with acid may be best accounted for by the fact that the fluted thickenings are high in pentosan content and consequently swell more severely in the upper regions where they are more pronounced. Thus the more uniformly thickened secondary wall regions of the lower half of the cell undergo less swelling because there is less material to be swollen and to be hydrolyzed by acid treatment. The fact that the swollen cellulosic walls may still show characteristic birefringence indicates that the crystallite structure correlates well with other information on the cellulosic-pentosan nature of this tissue. Possibly the cellulose molecules, being more slowly affected by the acid treatment, retain their orientation longer than do the pentosan units.

The "light line."—Various interpretations have been applied to the so-called "light line" of the macrosclereids in the integuments of the Leguminosae. One could easily gain the impression that it is a structural entity. Some authors have associated hardness and impermeability of the seed coat with the "light line " (Coe and Martin, 1920; see also Pammel, 1899; Hamly, 1932; and Zimmerman, 1936, for reviews of the literature). Among the earlier investigators, Marlière (1897) apparently arrived at the most logical explanation; he points out the structural relationships of the secondary walls, and their inner curved surface over the apex of each cell, to a simple phenomenon of light refraction. Lute (1928) has referred to this phenomenon as an "optical illusion." That the "light line" in *Melilotus alba* is a phenomenon of refraction, known in optical crystallography as the "Becke Line," has been conclusively demonstrated by Hamly (1935). This is exactly the situation with the macrosclereids of peas and beans, but, because the macrosclereids of peas do not possess "suberin caps" as found in *Melilotus,* it seems profitable to consider the "light line" in more detail as it appears in sections of the pea integument.

As already mentioned, the "light line" in the macrosclereids of peas is observed in close proximity to, or even at the same level with, the convergence of the thickenings over the apex of the lumen (fig. 3). Essentially the same situation is found in the macrosclereids of lima beans and other leguminous seeds. No pentosan test or other microchemical test differentiates this narrow band from the rest of the fluted thickenings. Optical characteristics show no difference in the structural orientation of the cellulosic fibrils other than change of position caused by the convergence of the thickenings over the apex of the lumen.

Some of the earlier authors have remarked that the "light line" is slow to absorb certain stains. It should be realized, however, that staining with most dyes, and particularly with very dilute solutions (as with methylene blue chloride) does not result in sufficient absorption to obscure the phenomenon of refractibility. This refraction may cause the narrow band to appear either more or less stained than the rest of the walls, depending upon the dye and its concentration. Variations in thickness of sections cut longitudinally through the macrosclereids influence the relative position and brightness of the "light line." In 5 μ sections this band sometimes is difficult to distinguish, and it appears much narrower than in thicker sections of three or four cell layers. In addition, the refractive indices of different mounting media influence both the appearance and the position of the "light line."

It is particularly interesting that in young macrosclereids where no thickenings of the secondary wall have developed the "light line" either does not appear or is difficult to distinguish. In comparing different ages of maturing cells, it was readily found that the more pronounced the development of secondary wall thickenings, the more pronounced the "light line." It is apparent and bright in the macrosclereids of fully matured, ripened peas.

In other seeds of the Leguminosae, as shown by Zimmerman (1936), it can be seen that the "light line" constitutes a similar characteristic of the wall thickenings of the macrosclereids. In some species these cells differ from those of peas in possessing domes, or "Spitzen," where the thickenings close over the apex of the lumen and underlie a subcuticular layer. In *Melilotus alba* these are described as "suberin caps" by Hamly (1932, 1935) and are considered by him to be the primary cause of impermeability in the seed coats of this species. He fur-

ther considers the "light line" to be caused by differences in refractive index between the composition of the suberin caps and the underlying walls of the macrosclereids. However, such "suberin caps" or special subcuticular layers are not found in peas and beans.

A plausible explanation, as indicated by Marlière (1897), is that the light striking the curved surfaces of the thickenings, where they converge over the apex of the lumen, is refracted to produce the "light line." In fact, one may produce a variety of longitudinally oriented "light lines" along the twisted thickenings of the secondary walls merely by making the necessary adjustments for different degrees of oblique illumination and, at the same time, may cause the transverse "light line" to disappear.

Although Hamly's (1935) interpretation of the "light line" is made upon a different basis, the optical principle is the same. In fact, one can interpret the Becke line in *Melilotus alba* as has just been suggested for beans and peas. Depending upon the mounting medium used, the differences in refractive index between the lumen and the wall thickenings may be greater than those reported by Hamly for the suberin caps and the secondary walls of the macrosclereids. It does not seem logical, therefore, in view of the natural optical properties, and the lack of chemical distinction of this line, to assign specialized functions of texture and permeability to an "optical illusion."

Osteosclereids.—It has already been mentioned that the radial, secondary wall thickenings of these cells give the same reactions with pentosan tests as do the fluted thickenings of the macrosclereids. Some authors have described the osteosclereids as lignified, but *Baptisia leucophaea* is the only species that Pammel (1899) lists in his tables as showing a positive reaction to the phloroglucinol test for lignin. Zimmerman (1936) lists no species showing a reaction for the "Tragerzellen."

With the possible exception of the hilum region, no lignin could be detected in this hypodermal layer in the integuments of young, maturing and ripe peas and lima beans. In the hilum region, where the osteosclereids become modified as previously described, some light pink color could be detected in the hypodermal modifications bordering the reticulate sclerenchymatous zone in older peas. Otherwise the cells of this layer give a more or less typical reaction to pentosan tests in their secondary walls, and even in the hilum region where their characteristic shape and secondary, radial wall structure are lacking, a general pentosan color reaction is obvious in the wall areas.

In close proximity to the hilum and the strophiole the osteosclereids in maturing peas display a more distinctive secondary wall development than elsewhere in the integument. They are more elongated and show a slightly fluted internal wall structure (fig. 16). As in the less pronounced secondary thickenings of the other osteosclereids, this wall structure is composed of a pentosan-cellulosic complex.

The fibrillar orientation of the cellulose of the curved, radial wall region of the osteosclereids likewise can be seen to be parallel with the long axes of the cells. The curved surfaces of these walls render interpretation of their optical characteristics difficult, but by careful observation while the stage of the microscope is slightly rotated to gain proper orientation of the curvatures with the first-order red plate, it can be verified that the fibrils maintain a lengthwise elongation as is further indicated by preparations treated with sulfuric acid. They show a polarization color of first-order gray which, with insertion of the first-order red plate with its slow ray parallel to the length of the cell, is raised to the second-order blue. This behavior demonstrates that the slow ray in the cell wall is lengthwise; that is, they have positive elongation.

Discussion.—As previously mentioned, seed coat texture is an important factor in utilization of peas in commercial food processing and preservation. The integument in many varieties is apt to become tough before the cotyledons have become hard or granular with starch. This problem of texture is particularly noticeable in the freezing preservation of peas. At about sieve size 4 most varieties begin to exhibit increased toughness. This size represents the stage at which the fluted thickenings of the macrosclereids have become pronounced (plate I). However, size may be used for maturity grading only within given varieties, since many varieties are characteristically large or small seeded. Peas of the more immature sizes in most varieties develop off-flavors, even in frozen storage.

Other problems of texture involve conditions of growth and harvesting and handling before the products are processed. Delay before freezing nearly always results in increased toughness of the integuments and produces other deleterious effects on quality. The rather prevalent opinion accepted by most investigators of the texture problem has been that the pectins of the middle lamellae and the chemical nature of the cuticle and "light line" are responsible for the characteristics of texture. Pectic changes may be involved in certain special conditions of growth, harvesting and handling, as discussed by Sayre, Willaman, and Kertesz (1931) in studies on the effects of nutrition upon texture.

It seems evident from the results of the pentosan tests and the various structural characteristics which have been described that texture and permeability are related to the pentosan-cellulosic complex of the secondary wall development of the macrosclereids of the integument. The osteosclereids undoubtedly contribute, but they do not form a compact tissue and structurally they may be considered of lesser significance. Not only do the secondary wall thickenings of these two tissues give positive pentosan reactions of qualitative specificity, but the middle lamellae also give some indication that "encrusting hemicelluloses" may increase during matu-

ration. Furthermore, the tentative identification of arabinose and xylose in extractions of the integuments strongly supports the histological interpretation of the pentosan complex and is in further agreement with other published analytical data on seed coats of beans and other angiosperms.

In some leguminous species the cuticle and subcuticular zones (Zimmerman, 1936) may also contribute to texture and impermeability of the integument, but no subcuticular zones are present in peas and beans of the common garden varieties, and the cuticle is less pronounced than in many other species. It is natural, however, that even a slight cuticle, even in immature tissues, will provide a barrier to the passage of water.

Quite obviously any practical method of pretreatment, designed to minimize the undesirable texture of inherently tough integuments of maturing peas intended for commercial freezing, would have little or no effect upon the pentosan-cellulose complex of the walls of the macrosclereids. Some effect might be achieved with the more easily altered fractions of the middle lamella pectins and perhaps the cuticle without impairment of other factors of quality, but these structures and components are not the major causes of tissue toughness.

An alternative which might eventually yield practical results of significance to food processing is the genetical development and selection of varieties with inherently more tender integuments at the age or sieve sizes considered most suitable for commercial food preservation. The histological information presented here strongly indicates the need for this more fundamental approach to some of the problems of quality and texture in commercially frozen foods.

SUMMARY

Histochemical methods of detecting pentosans in the walls of "hemicellulosic" tissues in seed plants were developed by adaptation of reagents that yield characteristic color products of condensation with pentoses. Differentiation between lignin and pentosan reactions was accomplished.

With these methods it was possible to detect the presence of pentosans in the secondary wall thickenings of the macrosclereids and the osteosclereids in the integuments of peas and lima beans. Also, color differentiation between these components and the polyuronides of the middle lamellae was obtained.

Arabinose and xylose were tentatively identified as sugars of the pentosan-cellulose complex of the secondary walls. Gelactose, gelacturonic acid and possibly arabinose apparently occur as constituents of the middle lamellae pectins.

The "light line" of the macrosclereids is considered to be merely a phenomenon of light refraction and its only significance to composition and textural characteristics of the maturing epidermis lies in the fact that it is caused by the thickened walls.

The crystallite orientation is parallel to the cellulosic fibrils in both the macrosclereids and the osteosclereids. Observations made on acid-swollen tissues are in agreement with optical properties as determined with polarized light. There also is agreement with the interpretations of the histochemical tests in relation to wall structure.

Further agreement exists between the observations and interpretations recorded here and other published information on the chemical composition, optical properties and structural characteristics of the walls of plant tissues.

The relationships of wall structure and texture to problems in commercial food preservation are briefly discussed. The tissue characteristics of texture and permeability are related to the pentosan-cellulose complex of the secondary walls in the macrosclereids. The cuticle also may affect permeability but bears little relationship to texture.

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EFFECTS OF VARIOUS CONCENTRATIONS OF BORON ON THE DEVELOPMENT OF TARAXACUM KOK-SAGHYZ IN SAND CULTURE [1]

Bernard S. Meyer[2]

SINCE THE first recognition of *Taraxacum koksaghyz* Rodin as an at least potentially important rubber plant, this species has been the subject of numerous investigations, particularly in Russia, but also in the last several years in other countries as well. The now extensive literature on kok-saghyz has been recently reviewed by Krotkov (1945). In a previous paper (Meyer, 1945), the effects of deficiencies of various essential mineral elements on the development of kok-saghyz have been discussed. In the present paper the results of an investigation of another phase of the mineral nutrition of this species—the effects of various concentrations of boron upon its development—are described.

The twofold effect of boron on plants, its essentiality in relatively small quantities, and its toxicity in relatively higher concentrations, is now so generally recognized that no detailed summation of the literature on this subject seems necessary. The earlier work on the role of boron in plants has been discussed by Brenchley (1927); later reviews are those of Brenchley (1936), McMurtrey (1938), Miller (1938), Willis (1939), and Hambidge (1941).

In agronomic and horticultural practice, the boron problem is encountered in some regions as an inadequate supply in the soil and in other regions as such a high concentration in the soil as to result in toxic effects on plants. Boron deficiency has been recognized in a number of species of crop plants in many parts of the world and may occur under a variety of different soil conditions. In some soils boron deficiency in plants results from an inadequacy of boron compounds in the soils; in other soils, particularly those which have an alkaline reaction or which have been heavily limed, boron may be pres-

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ent in adequate amounts but is rendered unavailable to plants. Suitable application of a boron fertilizer is necessary if maximum yields of many crops are to be attained on soils in either of these two categories. A number of serious "physiological diseases" of crop plants, the cause of which was formerly obscure, have in recent years been recognized as resulting from boron deficiency.

Over-fertilization with boron, on the other hand, may result in toxic effects on plants, the magnitude of such effects depending not only on the species of plant and the concentration of boron, but also on soil conditions. Toxic effects of boron have also been found in a number of species of plants when grown under irrigation in regions in which the supply of irrigation water contains appreciable concentrations of boron compounds. In southern California, for example, use of irrigation waters containing 0.5 p.p.m. or more of boron usually results in injury to certain crop plants, particularly walnut and citrus (Scofield and Wilcox, 1931).

Although the quantity of boron required is always low as compared with such elements as nitrogen, potassium, and phosphorus, it varies greatly from one kind of plant to another. Tolerance to excess quantities of boron also varies greatly from one species to another, in general being greater in those species having a high boron requirement. Of fifty species of plants tested, F. M. Eaton (1944) found that a considerable number made their greatest growth in sand culture when the solution used contained only a trace of boron. Some species, on the other hand, required a solution concentration of 10 to 15 p.p.m. of boron for maximum development, while numerous species were intermediate between these two extremes in boron requirement for best growth. The range of threshold concentrations for boron toxicity effects to become apparent showed a similar variation.

In "boron-sensitive" species the range between the quantities of boron necessary for normal development and those which induce toxic effects is very narrow. In lettuce, for example, optimal growth is obtained at 0.7 p.p.m., and injurious effects appear in the leaves at 0.9 p.p.m. (McHargue and Calfee, 1933). In some species foliar symptoms of boron toxicity may appear even at the boron concentrations most favorable to growth. This is true of the sunflower in which good growth unaccompanied by foliar symptoms of toxicity occurs at 0.5 p.p.m., but in which optimal development, measured as total green weight, occurs at 1.0 p.p.m., at which concentration symptoms of boron toxicity are clearly evident in the leaves (S. V. Eaton, 1940).

In contrast with those species which are extremely sensitive to boron, the more tolerant species often show moderately good, although not maximum development over a relatively wide range of boron concentrations both above and below the optimum.

EXPERIMENTAL METHODS.—The plants were grown in 8-mesh quartz sand,[3] three to each two-gallon pot, by a method of sub-irrigated sand cultures which has been previously used in other investigations on the mineral nutrition of kok-saghyz (Meyer, 1945). The nutrient solution used in all experiments was "complete" except that no boron was added, and contained the four principal salts in the following concentrations: 0.002 M KNO_3, 0.002 M KH_2PO_4, 0.003 M $Ca(NO_3)_2$, and 0.002 $MgSO_4$. To this solution was also added per liter 1 ml. of ferric tartrate solution and 1 ml. of a solution containing micrometabolic elements. The ferric tartrate solution contained 5 g. of $FeCl_3$ and 5 g. of tartaric acid per liter. The solution containing micrometabolic elements had the following composition: 1.8 g. $MnCl_2 4H_2O$, 0.1 g. $ZnCl_2$, 0.05 g. $CuCl_2 \cdot 2H_2O$, and 0.075 g. MoO_3 per liter. This complete solution had a calculated osmotic pressure of about 0.5 atm., and an initial pH of 4.0. Boron was introduced into the solutions as H_3BO_3 by adding the requisite volume of a standard solution containing 1 mg. of boron per milliliter of solution before making up to final volume. Boron concentrations are expressed as parts per million of the element. Introduction of even the highest concentrations of H_3BO_3 used did not depress the initial pH of the solution as compared with the basic solution by more than 0.2 unit. While being used to irrigate the plants, a given volume of solution steadily increased in pH value, but values higher than 6.8 were not attained in any culture at any time during the investigation. A laboratory grade of distilled water was used in making all solutions.

The three experiments reported in this paper were performed only after the results of several orientation experiments had indicated that kok-saghyz is a plant with a relatively high boron requirement. There were six cultures in each experiment. The concentrations of boron used were 0, 5, 10, 15, 20, and 25 p.p.m. Since no effort was made to exclude minor sources of boron contamination from reagents, water, sand, or pots in those cultures to which no H_3BO_3 was added, these cultures should, strictly speaking, be designated as trace boron cultures. The seed used was from the 1942 crop of kok-saghyz grown at St. Paul, Minnesota. Seed was sown directly in the sand-filled pots and the resulting stand of seedlings was gradually thinned until only three well-spaced plants remained in each pot. For approximately the first two weeks of their existence the seedlings were watered with distilled water applied as a fine spray, and for approximately the next two weeks they were also sprayed occasionally with the basic solution, previously described, at half strength. When the plants were about one month old, irrigation with full strength basic solution was started, 3.5 liters of solution being used in each culture set-up of pot plus reservoir bottle. After about two weeks this solution was replaced with a fresh solution to which H_3BO_3 had been added in suffi-

[3] Pennsylvania Glass Sand Co., Lewistown, Pennsylvania.

TABLE 1. *Fresh weights and dry weights in grams, and percentage rubber contents on a dry weight basis of the roots of kok-saghyz plants grown in solutions containing different concentrations of boron.*

Expt. no.	Duration of experiment	Boron concentration in parts per million															
		Trace			5			10			15			20			25	
		Fresh wt. roots	Dry wt. roots	Rubber %	Fresh wt. roots	Dry wt. roots	Rubber %	Fresh wt. roots	Dry wt. roots	Rubber %	Fresh wt. roots	Dry wt. roots	Rubber %	Fresh wt. roots	Dry wt. roots	Rubber %	Fresh wt. roots	Dry wt. roots	Rubber %
1	1/8/44–7/9/44	34.3	7.19	1.25	24.2	4.81	1.65	47.3	9.25	1.50	51.0	11.15	1.90	48.5	9.84	1.35	35.2	5.97	1.05
2	3/30/44–10/7/44	47.4	8.98	1.20	49.4	8.48	0.75	62.1	12.14	2.85	36.9	6.40	1.05	32.1	4.93	1.95	35.2	6.12	1.80
3	6/10/44–12/15/44	18.5	3.74	0.60	32.3	7.44	2.30	30.5	5.71	2.50	35.6	6.03	1.60	32.3	4.21	0.90	25.1	3.89	1.35
Average weight per plant		11.1	2.21		11.8	2.41		15.5	3.01		13.7	2.62		12.5	2.11		10.6	1.78	
Average per cent rubber				1.02			1.57			2.98			1.52			1.40			1.40

cient quantity to each reservoir to bring the concentration of boron up to the desired value for that solution. The cultures were irrigated once, twice, or three times per day, depending upon prevailing climatic conditions. Water lost in evaporation and transpiration was compensated for by adding distilled water until the solution was brought to its initial level in each reservoir bottle before each irrigation. About once every two weeks the solutions in use were discarded and replaced with entirely fresh ones.

From about the first of June until about the middle of October the cultures were kept outdoors. The high temperatures of mid-summer in the Ohio climate are not the most favorable for the development of kok-saghyz plants. Excellent growth was obtained, however, by shading the plants with a layer of cheesecloth and by so situating the cultures so that they did not receive full insolation until mid-morning (fig. 1). Prior to early June and after the middle of October, the cultures were kept in a greenhouse in which the attainment of high temperatures on bright days was avoided as far as possible by suitable ventilation. The relative position of the pots in each experiment was shifted according to a randomizing scheme each time the solutions were changed in order to minimize the possible effects of an asymmetric distribution of light or any other environmental factor.

The duration of each experiment from the time of sowing the seed to the time of harvesting the roots was about six months, this being the approximate length of the growing season when kok-saghyz is grown as an annual crop. Upon dismantling each experiment all leaves were plucked from the plants. Such defoliated plants are henceforth referred to simply as "roots." The entire root system of each plant plus the short stem constituted the material used in all determinations. The fresh and dry weights of each individual root were determined. In the determination of the dry weights, roots were dried at 70°C. for about 48 hours. All root samples were also analyzed for their percentage rubber content.[4]

RESULTS AND DISCUSSION.—Data on the fresh and dry weight yields of the roots and their percentage rubber contents for the three experiments are summarized in table 1. All fresh and dry weight values as given in this table, except averages, are aggregate values for the three plants in each pot. There was not a sufficient number of replications to admit of statistical treatment, but the trend of the results points to some unmistakable conclusions. Highest average yields of roots, on either a fresh weight or a dry weight basis, were gotten at 10 and 15 p.p.m. of boron, lesser yields being obtained at both lower and higher concentrations. These results indicate that the maximum development of kok-saghyz plants

[4] Rubber analyses were made at the Laboratory of Rubber Investigations, Plant Industry Station, Beltsville, Maryland, by a turbidimetric method which has not been published.

results only when the concentration of available boron compounds is much higher than is required for the best growth of many other species. As previously mentioned, optimal development of many species occurs at boron concentrations of 1 p.p.m. or less in sand culture. While a number of species are known to have a higher boron requirement than this for maximum growth, only a few are definitely known to have a boron requirement as high as that of kok-saghyz. Among the better known examples of species of which this is true are sugar beet, cotton, and asparagus.

In physiological consonance with its high boron requirement kok-saghyz also has a high boron tolerance. Even at a solution concentration of 25 p.p.m. development of the plants was in no observable way abnormal (fig. 1), and the yield of roots was about two-thirds the maximum yield obtained at 10 or 15 p.p.m. of boron. F. M. Eaton (1944) classified the fifty species of plants with which he worked into three groups, boron-sensitive, boron-semi-tolerant, and boron-tolerant plants, the last group comprising only nine species. If its relative boron tolerance is computed according to Eaton's procedure, kok-saghyz falls into the group of boron-tolerant plants.

Although the optimum boron concentration for this species in sand culture appears to be about 10–15 p.p.m., it is noteworthy that moderately good growth is obtained not only at concentrations considerably greater than this, but also at concentrations considerably less than this. Even in the trace boron cultures the fresh and dry weight yields of

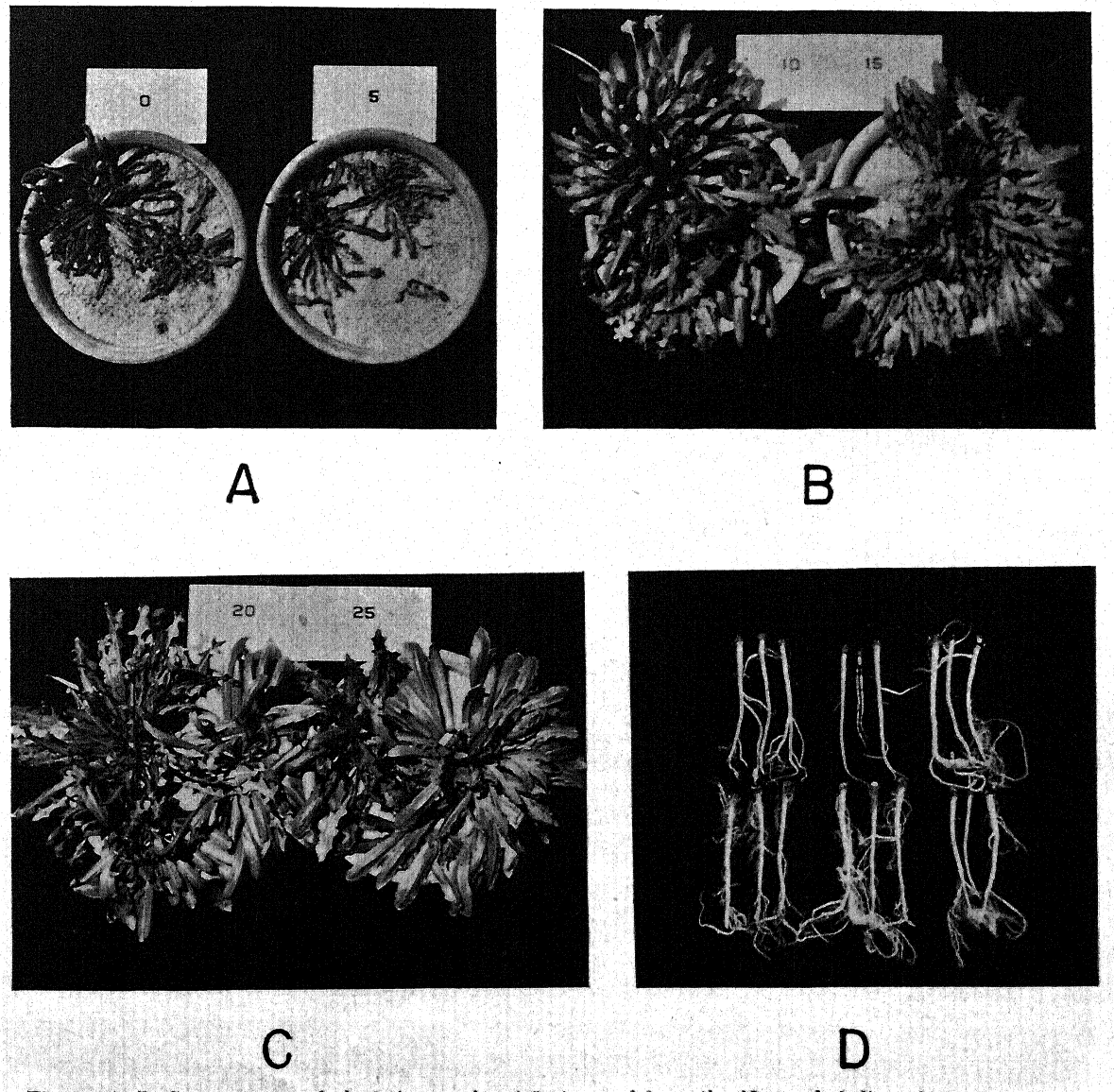


Fig. 1.—A, B, C, appearance of plants in experiment 1 at age of 6 months. Numerals indicate boron in p. p. m.—D, appearance of roots from same plants. Left to right, top row, 0, 5, 10 p. p. m., bottom row, 15, 20, 25 p. p. m. of boron.

roots are, on the average, about two-thirds as great as at the most favorable boron concentrations. It seems evident that, like other boron tolerant species, kok-saghyz grows moderately well over a relatively wide range of boron concentrations, even though optimum development may occur only within a much narrower range.

The trend of the results shown in table 1 suggests the possibility that the concentrations of boron most favorable to the development of roots are also most favorable to the accumulation of rubber in the roots. The small number of replications, however, in conjunction with the considerable variability in the percentage rubber contents at any one boron concentration, and the small differences in rubber content from one boron concentration to the next preclude any definite conclusion on this point.

It is interesting to compare the results of this investigation on kok-saghyz with that of Mitchell *et al.* (1944) on the boron relations of guayule (*Parthenium argentatum* Gray), another rubber-bearing species of composite. The boron requirement of guayule apparently is considerably less than that of kok-saghyz. Although guayule plants were found to tolerate considerably higher concentrations without excessive retardation of growth, optimum vegetative development occurred only within the relatively narrow and low range of 0.1–2 p.p.m. in gravel culture. Maximum accumulation of rubber was also found to occur in the same range of boron concentrations.

In addition to a reduction in total root growth, certain other symptoms of boron deficiency in kok-saghyz appear to be characteristic. In general there were fewer fibrous roots on plants which received relatively low concentrations of boron (fig. 1). Foliar symptoms of boron deficiency in kok-saghyz were apparent in many but not all plants receiving 5 p.p.m. of boron or less (fig. 1). Such symptoms usually appeared only rather late in the growth cycle and consisted in a retardation in growth and a distortion of the younger leaves, accompanied by a necrosis which gradually spread from the tip towards the base until the entire leaf was dead.

The only observed effects of superoptimum concentrations of boron were a reduced yield of roots and usually a somewhat, although not markedly, diminished development of foliage. Discolorizations and other foliar symptoms of boron toxicity which have been described for a number of species were not apparent in any of the kok-saghyz plants receiving excess boron. A probable explanation of this fact is that there is a regular, progressive dying off of the older leaves of a kok-saghyz rosette even under the most favorable growth conditions. Since boron toxicity symptoms characteristically appear first in the older leaves of a plant, distinctive visible evidences of physiological disturbances resulting from excess boron either did not develop or else were obscured by this regularly occurring death of the leaves.

The findings of this investigation have certain agronomic implications for kok-saghyz as a crop plant. As is well known, the supply of boron in many soils is inadequate, particularly for crops such as kok-saghyz which have a relatively high boron requirement. Some fertilization with boron would appear to be indicated if a maximum yield of kok-saghyz is to be obtained on many soils. It seems very likely that the yields of many of the test plantings of kok-saghyz which have been made during the past few years in various parts of the world would have been improved if a boron fertilizer had been applied in suitable quantities. On the other hand, its high boron tolerance makes it less likely than many others that this species will suffer injury from injudiciously high applications of boron. The high boron tolerance of kok-saghyz also indicates that it could be grown in regions where irrigation water contains appreciable concentrations of boron compounds with much less likelihood of injury than many other species.

SUMMARY

An investigation was made of the effects of various concentrations of boron on the development of kok-saghyz in sand culture. Maximum root yields were obtained at 10–15 p.p.m. of boron, although moderately good development of the plants occurred over a considerably wider range of boron concentrations. Kok-saghyz thus has a considerably higher boron requirement and tolerance than most other species which have been investigated. Foliar symptoms could be recognized for boron deficiency but not for boron toxicity. The results also suggest that the same boron concentrations which favor maximum root development also result in the highest percentage rubber contents in the roots, but the data on this point are not conclusive.

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THE INHIBITING EFFECT OF THE TERMINAL BUD ON FLOWER FORMATION IN THE AXILLARY BUDS OF THE HADEN MANGO (MANGIFERA INDICA L.)[1]

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UNPUBLISHED INVESTIGATIONS by the senior author on floral differentiation in the Haden variety of mango at Orlando, Florida, show that in this variety differentiation of the inflorescence begins within a very short period before the expansion of the terminal buds in December to February, and the process continues during the period of bud expansion.

Inflorescences in the mango are determinate and appear under normal conditions only from terminal buds. Axillary buds usually remain dormant during the flowering period, and histological examination of these buds during this period showed only a meristematic growing point and several bud scales. These buds remain dormant during the development of the terminal inflorescences and, if fruits are set, during the development of the fruit. After the fruit is harvested, some of the axillary buds expand into vegetative shoots.

It was observed, however, that if the terminal bud was cut off during or just before the flowering period, axillary buds promptly differentiated into inflorescences and produced panicles of flowers within six weeks after the removal of the terminal bud. Since floral primordia were formed in the axillary buds of only those shoots from which terminal buds were removed, it follows that the terminal bud must have inhibited differentiation in the axillary buds.

In an experiment designed to study the nature of this inhibitory effect, terminal buds were removed from 120 branches. Sixty of these were ringed about 2 feet (20 to 30 nodes) below the distal end to prevent the transmission of any flowering substance from the leaves on the branch below the ring to the axillary buds above the ring. The stem portions above the ring of the ringed branches and the comparable distal 2 feet of the non-ringed branches were defoliated at various intervals after decapitation. Some were defoliated immediately after decapitation, others after intervals of four and eight hours and 1, 4, 8, and 28 days. On a few ringed branches one leaf was left above the girdle for the duration of the experiment.

Floral differentiation occurred in some of the axillary buds on each branch after decapitation in all non-ringed treatments, and in all ringed groups if the leaves were left on four days or more. On ringed branches that were defoliated immediately or within 24 hours after decapitation, only vegetative shoots were produced by the axillary buds. However, if one leaf was left on the stem above the ring, all of the buds that grew out (three to eight per branch) produced inflorescences.

These results indicate that floral induction had not occurred in axillary buds before the terminal buds were removed, and that floral induction took place in the axillary buds in a period of between one and four days after the removal of the terminal buds from leafy, ringed shoots. On non-ringed branches that had been defoliated for 20 to 30 nodes back from the distal end, flowers were initiated in axillary buds on the distal end of the defoliated region. Therefore, it is evident that the flowering substance may be transmitted from the leaves through a distance of 20 to 30 nodes to the buds of the defoliated region.

Hamner and Bonner (1938) in describing flowering in the cocklebur have used the term "induction" to refer to the changes, probably chemical, within the plant, that result in the differentiation of floral primordia. In the mango shoots of this experiment the initiation of floral primordia in the axillary buds was shown to be promoted by the leaves and inhibited by the terminal bud. It seems probable that, in the presence of the terminal bud, induction as well as differentiation was in some manner inhibited. If floral induction had been effected in any of the axillary buds before the terminal bud was removed, it would have to be assumed either that all buds so affected remained dormant or else that induction is in some way reversible, since the axillary buds which grew on the ringed shoots that were defoliated with-

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in 24 hours after decapitation produced only vegetative shoots. Neither of these two assumptions seems plausible. Therefore, although the evidence is not conclusive that induction as well as differentiation was inhibited, it seems probable that induction did not take place when the axillary buds were dormant even in the presence of leaves, and it is possible that induction can take place only when cell division has started. The flower-inducing substance may play no part in the initiation of growth, but when present in sufficient amounts at the beginning of growth, it probably determines the course of differentiation of the tissue in the axillary buds. When the inhibiting effect of the terminal bud was removed, growth began in the axillary buds; if leaves were present on the stem, induction and differentiation of flowers rapidly followed.

SUMMARY

Inflorescences in the mango are determinate and appear under normal conditions only from terminal buds. If, however, the terminal buds are removed during the flowering period, inflorescences are produced by axillary buds in the distal region of the shoot. Normally the axillary buds remain dormant during this period, but later some of them produce vegetative shoots. Results of experiments on girdled, decapitated branches, which were defoliated at various intervals after the removal of the terminal bud, indicate that when leaves were present above the girdle, floral induction took place in the axillary buds in a period between one and four days after decapitation and that floral differentiation rapidly followed.

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SPECIFICITY OF POTASSIUM AND MAGNESIUM FOR GROWTH OF ASPERGILLUS NIGER [1]

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Reports indicating or denying replaceability in some degree of essential by unessential chemical elements in the nutrition of fungi and green plants have appeared frequently—Sestini (1891), Benecke (1895), Lehr (1941), van Itallie (1943). Proof of the existence of biological or "partial" substitution should exclude any possibility of positive results due to amelioration of environmental conditions. The question concerns itself with the ability of an unessential element to participate in the fundamental reactions and composition of compounds comprising the basic activities of the plant cell.

The experimental data herein recorded deal primarily with a study of the factors influencing *experimental* biological substitution. The fungus, *Aspergillus niger* van Tiegh, was used as the test organism. Despite former claims to the contrary, its mineral requirements agree well with those of the green plant, except that calcium and boron are not needed. Agreement in yields between duplicate cultures can be held to better than ± 5 per cent in 95 per cent of all determinations with this organism (Steinberg and Bowling, 1939), an important consideration.

The elements most similar to each other in chemical properties are the alkali metals (Li, Na, K, Rb, Cs), and the alkaline-earth metals (Be, Mg, Ca, Sr, Ba). Of these only potassium and magnesium are essential for nutrition of *Aspergillus*. Replaceability of these two elements by others of their respective series was studied in the dibasal nutrient solution (Steinberg, 1945). This nutrient solution contained each essential element required by the fungus in *minimal quantity for maximum growth*. A decrease in any mineral component of this solution resulted in decreased yields, whereas moderate increases in any or all were without effect. Adjustment in external buffering was not a factor in yield since mineral absorption is complete and much free organic acid is formed.

Experimental methods.—*Aspergillus niger* van Tiegh was grown on 50 cc. of a dibasal solution in 200 cc. flasks at 35°C. for four days. Quartzware only was used. The dibasal solution contained: Water (triply distilled) 1,000 cc., sucrose (0.00087 per cent ash) 50 g., NH_4NO_3 1.90 g., K_2HPO_4 0.35, $MgSO_4 \cdot 7H_2O$ 0.25 g. Fe, Zn, Cu, Mn, Mo, and Ga were added in quantities of 0.40, 0.40, 0.10, 0.10, 0.02, and 0.02 mg. per liter, respectively, as the chlorides. It was neutral in reaction. The salts were reagent chemicals; the micronutrients were practically pure spectroscopically, as may be noted in the footnote of table 1. The minus potassium solution contained 0.27 g. $(NH_4)_2HPO_4$ instead of K_2HPO_4. The minus magnesium solution contained 0.12 g. $(NH_4)_2SO_4$ instead of 0.25 g. $MgSO_4 \cdot 7H_2O$. Reagent KCl and $MgCl_2 \cdot 6H_2O$ were employed in determining growth concentration values. The chlorides of substituents (Li, Na, Rb, Cs, Be, Ca, Sr, Ba) were almost pure spectroscopically except beryllium which contained probably less than 0.0001 per cent magnesium.

The dibasal solution contained (Steinberg and Bowling, 1939) per liter: N 665 mg., K 157.2 mg., P 62.3 mg., Mg 24.8 mg. and S 32.5 mg. of which the fungus absorbed 98.0, 86.5, 96.1, 46.4, and 70.9 per

[1] Received for publication December 10, 1945.

TABLE 1. *Effect of homologous chemical elements on growth of Aspergillus niger at different levels of potassium nutrition in a dibasal solution.*

Potassium concentrations	Control	50.0 mg. lithium ion per liter	50.0 mg. sodium ion per liter	50.0 mg. rubidium[a] ion per liter	50.0 mg. cesium[b] ion per liter
mg. per liter	milligrams	milligrams	milligrams	milligrams	milligrams
15.0	284.5	280.3	362.2	321.5	293.3
30.0	464.4	459.6	588.1	425.1	521.1
45.0	605.4	479.4	671.7	592.1	637.2
60.0	698.5	709.6	782.7	695.7	745.3
75.0	826.1	783.5	889.2	807.1	803.6
90.0	887.2	948.8	852.1	904.1	834.6
105.0	958.2	959.8	888.4	927.0	828.7
120.0	989.2	925.8	904.5	946.7	888.5
135.0	1039.9	997.6	956.5	971.2	870.5
150.0	1044.9	1050.1	1007.7	955.2	838.5

[a] The rubidium contained 0.007 per cent or 0.0035 mg. sodium; spectroscopic purity 99.94 per cent.
[b] The cesium contained 0.06 per cent or 0.03 mg. sodium; spectroscopic purity 99.9 per cent.

cent, respectively, in reaching maximum growth. Maximum yield usually varied between 1,150 mg. and 1,250 mg. per 50 cc. with this solution.

The procedure followed in testing for specificity was based on the determination of quantity-yield values for potassium or magnesium in the absence or presence of each of the possible substituents. The potassium and magnesium values with the dibasal solution were based on triplicate runs, i.e., an average of six cultures, and the sodium and beryllium values on duplicate runs, i.e., an average of four cultures. Other values for yield were the average of two cultures.

It will be observed that the maximum quantities of potassium and magnesium employed in this study were 150 mg. of potassium per liter and 10 mg. of magnesium per liter. Many trials with greater quantities of K and Mg over an interval of years have shown that the maximum possible yield under these conditions varied from 1,050 to 1,200 mg. Preliminary tests with an excess of potassium or magnesium not included in these data further substantiated these observations.

EFFECT OF HOMOLOGOUS ELEMENTS ON YIELDS WITH POTASSIUM.—The alkali metals include lithium, sodium, potassium, rubidium, cesium and an element of atomic number 87. The members of this homologous series and their chemical compounds have been found quite uniform in their chemical and physical properties. Only sodium and potassium are definitely known to be essential to organisms, though varying claims have also been made with respect to the others.

The quantity-yield determinations for potassium in the absence and presence of each of the other members of this homologous series are given in table 1. The minimum potassium concentration for maximum growth, as mentioned in the section on experimental methods, is 157.2 mg. per liter of which 86.5 per cent is absorbed (dibasal solution).

TABLE 2. *Effect of homologous chemical elements on growth of Aspergillus niger at different levels of magnesium nutrition in a dibasal solution.*

Magnesium concentrations	Control	5.0 mg. calcium ion per liter	25.0 mg. calcium ion per liter	5.0 mg. beryllium[a] ion per liter	5.0 mg. strontium ion per liter	5.0 mg. barium ion per liter, sulfate –S	5.0 mg. barium ion per liter, sulfite –S
mg. per liter	milligrams	milligrams	milligrams	milligrams	milligrams	milligrams	milligrams
1.0	131.0	99.9	120.8	84.7	103.0	113.4	104.5
2.0	247.1	219.3	274.6	280.2	266.9	265.2	281.8
3.0	461.6	399.9	414.4	507.6	420.2	413.9	445.1
4.0	636.4	497.6	574.6	779.3	617.3	601.7	602.3
5.0	762.9	805.1	747.4	913.1	790.4	724.2	804.5
6.0	906.2	859.8	895.9	1012.2	863.9	825.8	860.9
7.0	1011.2	961.0	898.1	985.9	854.3	880.6	917.7
8.0	1058.8	987.9	961.7	1040.2	887.9	912.6	944.2
9.0	1077.8	977.1	957.7	1050.0	936.1	1003.0	950.0
10.0	1087.7	974.2	928.8	1023.7	902.2	933.0	967.7

[a] Beryllium contained a minute quantity of magnesium, probably less than 0.0001 per cent.

TABLE 3. *Effect of homologous chemical elements on growth of Aspergillus niger at different levels of potassium and magnesium nutrition in a solution containing double dibasal quantities of macronutrients.*

Potassium per liter	Yields with potassium in the presence of its chemical homologues (50.0 mg. per liter)					Magnesium per liter	Yields with magnesium in the presence of its chemical homologues (5.0 mg. per liter)				
	Control	Lithium	Sodium	Rubidium	Cesium		Control	Beryllium	Calcium	Strontium	Barium
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
15.0	256.3	221.1	401.3	332.5	227.5	1.0	103.5	60.8	138.1	134.0	98.8
30.0	446.1	418.1	783.1	748.1	517.6	2.0	296.9	228.4	322.9	252.5	235.8
45.0	641.2	583.3	896.7	923.1	740.2	3.0	321.3	422.5	410.7	477.1	403.2
60.0	823.4	689.2	1042.0	980.2	854.7	4.0	445.8	669.7	555.3	588.8	513.0
75.0	955.2	775.9	1089.0	1068.6	994.2	5.0	648.0	818.6	682.8	741.4	796.2
90.0	988.0	793.0	1070.0	1097.9	1110.7	6.0	660.9	869.6	925.8	931.3	745.4
105.0	1065.2	964.0	1093.1	1129.1	1110.1	7.0	826.5	1049.6	1089.6	871.3	859.6
120.0	1059.2	1010.7	1095.5	1127.9	1125.9	8.0	884.6	915.0	1131.1	989.0	1034.1
135.0	1113.9	979.9	1084.9	1135.9	1094.3	9.0	903.5	1018.6	1111.7	1007.9	993.1
150.0	1145.9	1098.9	1146.5	1153.6	1234.2	10.0	895.7	988.3	1211.7	1170.7	1111.8

Growth with increasing quantities of potassium was benefited only by the presence of sodium, and then only when potassium deficiency was 50 per cent or more of that required for maximum yield. These increases in yield did not occur with lithium, rubidium or cesium. All, including sodium, displayed a slightly harmful effect at potassium concentrations approximately optimum for growth. This inhibition in rate of growth appeared to increase with increased atomic weight of the element, being least for lithium and greatest for cesium. Maximum growth required 150 mg. potassium per liter.

EFFECT OF HOMOLOGOUS ELEMENTS ON YIELDS WITH MAGNESIUM.—The alkaline-earth metals include beryllium, magnesium, calcium, strontium, barium and radium. The members of this homologous series, particularly magnesium, calcium, strontium, and barium, are also quite uniform in their chemical and physical properties. Only magnesium and calcium are essential to organisms, though here again varying claims can be found in the literature with respect to the others.

The quantity-yield determinations for magnesium in the absence and presence of each of the other members (except radium) of this homologous series are given in table 2. The minimum magnesium concentration for maximum growth, as already mentioned, is 24.8 mg. per liter of which 46.4 per cent is absorbed by the organism (dibasal solution).

Growth with increasing quantities of magnesium was benefited only by the presence of beryllium, and then only when magnesium deficiency was about 50 per cent or more of that required for maximum yield. Calcium, strontium, and barium did not give increases in yield. All, including beryllium, brought about slight decreases in yield at magnesium concentrations approximately optimum for growth. The effect of the presence of barium on yields was also tested with sulfur present as sulfite to guard against low yields due to locking of sulfur as barium sulfate. The growth effect of calcium at a higher concentration (25 mg./l) was found to be quite similar to that obtained at the lower concentration (5 mg./l). Maximum growth required 10 mg. magnesium per liter.

TABLE 4. *Effect of sodium and beryllium on growth of Aspergillus niger at different levels of potassium and magnesium nutrition, respectively, in a solution containing double dibasal quantities of micronutrients.*

Potassium per liter	Yields with potassium in presence of sodium and ×2 micronutrients		Magnesium per liter	Yields with magnesium in presence of beryllium and ×2 micronutrients	
	Control	Sodium (50 mg./l)		Control	Beryllium (5 mg./l)
milligrams	milligrams	milligrams	milligrams	milligrams	milligrams
15.0	358.5	506.3	1.0	131.8	103.5
30.0	501.3	815.5	2.0	297.7	312.2
45.0	754.1	881.7	3.0	569.3	560.2
60.0	867.5	914.6	4.0	760.2	783.4
75.0	909.8	961.3	5.0	880.3	936.3
90.0	914.0	972.5	6.0	975.4	999.7
105.0	973.4	978.0	7.0	1027.7	1017.6
120.0	991.7	1005.3	8.0	1042.5	1018.6
135.0	998.2	985.5	9.0	1051.3	1042.3
150.0	996.5	1042.0	10.0	1064.0	1067.2

TABLE 5. *Effect of homologous elements on growth of Aspergillus niger at two levels of potassium and magnesium nutrition in an acid dibasal solution.*

Element added (50 mg./l)	Yields in an acid solution pH = 3.18		Element added (5 mg./l)	Yields in an acid solution pH = 3.27	
	75 mg. K/l	150 mg. K/l		5 mg. Mg/l	10 mg. Mg/l
	milligrams	milligrams		milligrams	milligrams
None	886.4	1061.1	None	900.7	1071.7
Li	970.1	1052.9	Be	854.2	1066.5
Na	1014.2	1113.8	Ca	931.2	1090.3
Rb	958.7	1051.4	Sr	978.0	1107.4
Cs	1045.8	1101.8	Ba	962.8	1070.2

RELATION OF EXCESS MACRONUTRIENTS TO SUBSTITUTION.—The data of table 3 are a repetition of those in the previous tables and were obtained in an entirely similar manner, except for a doubling of the salt concentrations. Micronutrient and substituent concentrations were unaltered. The quantities of potassium and magnesium required for maximum growth were again 150 and 10 mg. per liter, respectively. Presence of double the required quantities of other macronutrients had a beneficial influence on yields in the presence of substituents. That is to say that sodium, rubidium and cesium gave increases in yield when potassium was deficient in quantity, as did beryllium, calcium, strontium, and barium when magnesium was deficient. Sodium and beryllium proved on the whole most effective as substituents in their respective series. It should also be noted that, whereas excess macronutrients did not diminish yields in the control with potassium, the reverse was true for yields in the control with magnesium.

RELATION OF EXCESS MICRONUTRIENTS TO SUBSTITUTION.—The effects of a moderate excess of micronutrients on substitution are shown in table 4. Maximum yields with potassium were slightly diminished but the potassium/sodium effect persisted. The magnesium/beryllium effect on yields did not appear in the presence of excess micronutrients. Another difference consisted in the enhanced yields with lower quantities of potassium, an effect not shown by magnesium. The optima for potassium (150 mg./l) and magnesium (10 mg./l) were not altered by an excess of micronutrients.

RELATION OF INCREASED ACIDITY TO SUBSTITUTION.—The effect of increased acidity on yields with potassium and magnesium in the presence of their homologues is summarized in table 5. The indicated acidities were obtained through addition of hydrochloric acid to the practically neutral dibasal solution prior to sterilization. Formation of free organic acid takes place during growth, acidities of pH 1.3 having been recorded. Increases with homologues were general at the lower concentration of potassium, whereas only slight or no increases occurred under similar conditions with magnesium. Maximum yields were not increased at optimum concentrations of potassium and magnesium by addition of their respective homologues. The optima for growth of potassium and magnesium were 150 and 10 mg. per liter, respectively.

RELATION OF INCREASED INOCULUM TO SUBSTITUTION.—Table 6 contains a tabulation indicating the effect of size of inoculum on substitution. Spore dosage is characterized as low, moderate and high. It is evident that with increase in quantity of spores per culture the effects of sodium and beryllium in remedying deficiencies of potassium and magnesium, respectively, tend to lose their specificity. A possible explanation is that with increased inocula the growth for each spore diminishes proportionally and, therefore, also the respective potassium and magnesium deficiencies. The increase in mineral elements added with increased number of spores could, however, also account in part for the effect observed. In each case 150 mg. of potassium per liter and 10 mg. of

TABLE 6. *Effect of homologous elements on growth of Aspergillus niger at two levels of potassium and magnesium nutrition as related to size of inoculum.*

Element added (50 mg./l)	Yields with potassium				Element added (5 mg./l)	Yields with magnesium			
	75 mg. K/l		150 mg. K/l			5 mg. Mg/l		10 mg. Mg/l	
	Moderate inoculum	High inoculum	Moderate inoculum	High inoculum		Low inoculum	High inoculum	Low inoculum	High inoculum
	mg.	mg.	mg.	mg.		mg.	mg.	mg.	mg.
None	830.7	890.6	1021.3	1065.7	None	708.5	774.2	(1027.4)	1069.4
Li	856.9	880.6	1020.1	1020.2	Be	830.3	978.0	979.2	1055.7
Na	958.6	1001.8	1058.0	1070.5	Ca	647.2	862.3	920.0	1035.2
Rb	906.8	972.8	1031.3	1053.1	Sr	661.3	881.2	933.1	(936.4)
Cs	943.2	960.9	1088.6	1084.5	Ba	669.8	910.4	948.2	1058.2

magnesium per liter sufficed for maximum growth.

Discussion of results.—Addition of sodium to cultures markedly deficient in potassium brought about increased yields under all conditions. Specificity in supplementation of potassium deficiency was most marked in the unaltered dibasal solution. Moderate increases in macronutrients, micronutrients (?), acidity, and size of inoculum also led to increased yields with other elements (Li, Rb, Cs) of this series. In addition, these variations in themselves led to increased yields when potassium was deficient. Addition of beryllium to cultures deficient in magnesium brought about increased yields except with increased micronutrients and increased acidity. Each modification (increased micronutrients were not tested) of the dibasal solution led to increased yields with other elements (Ca, Sr, Ba) of this series. Increased micronutrients and acidity also increased yields when magnesium was deficient in quantity for maximum growth. Conditions causing loss in specificity of results with sodium gave a similar response with beryllium so far as tested. Excesses in each instance destroyed specificity of effect. These generalized responses with the elements of the two series are interpreted as probably compensatory (ion antagonism) and as tending to neutralize the effects of excesses. They are not considered to bear any direct relation to the phenomenon of biological substitution of a nutritive element.

The results obtained on supplementation of deficiencies in potassium or magnesium in the dibasal solution with sodium and beryllium, respectively, disclose a definite specificity in effect with each series. They do not, however, afford any actual evidence for substitution in the organism, if specificity be excluded as proof. Such substitution is, however, not unlikely since minimum quantities are concerned, and absorption of potassium and magnesium is known to be almost complete under these conditions.

There exist also regular differences in the behavior of sodium and beryllium in these experiments. The magnesium/beryllium effect disappears with an increase in micronutrients and with an increase in acidity. Maximum yields with optimum potassium or magnesium were in no instance increased by additional macronutrients, micronutrients, acidity, or inoculum. Intermediate yields, however, were increased in each case with potassium, but only with excess micronutrients or acidity with magnesium. Those conditions causing elimination of the magnesium/beryllium effect were those capable of bringing about increased efficiencies in utilization of magnesium at intermediate yields.

The theoretical implications of these differences in the potassium/sodium effect and the magnesium/beryllium effect are still obscure on the basis of these data. As yet unpublished data indicate, however, a definite correlation to the optima of potassium and magnesium for maximum growth in the dibasal solution. The optimum for potassium in the dibasal solution had been found to be 157.2 mg. per liter and of magnesium 24.8 mg. per liter. The growth curves herein presented indicated potassium gave a maximum yield with 150 mg. per liter, and magnesium with 10 mg. per liter.

SUMMARY

Aspergillus niger van Tiegh. was grown in a dibasal solution (optimum yield solution of minimum salt content) for four days at 35°C. Sodium and beryllium gave increased yields with deficiencies in potassium and magnesium, respectively ("biological substitution"). Increased macronutrients, micronutrients, acidity, or inoculum did not eliminate the potassium/sodium effect, but did cause increased growth with extreme deficiencies of potassium and caused a loss of specificity in action of sodium. That is, positive effects were also caused by other alkali metals (Li, Rb, Cs). The potassium optimum (150 mg./l) remained unaltered in all instances. Responses in the magnesium/beryllium effect to these variations in the dibasal solution were similar (Ca, Sr, Ba), except for its elimination in the presence of increased micronutrients and of acidity. Increased macronutrients or inoculum did not lead to increased yields with extreme deficiencies in magnesium. The magnesium optimum (10 mg./l) for maximum growth remained unaltered in all instances. Biological pseudosubstitutions (Li, Rb, Ca, Sr, Ba) were attributed to amelioration of inhibitions (ion antagonism).

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GENERAL SECTION

(Abstracts of this Section are arranged alphabetically according to author)

Abbe, E. C., and M. Pollock, University of Minnesota, Minneapolis, Minn. *The interrelation between the rate of organ growth and the rates of multiplication and enlargement of its constituent cells in Anacharis* (*Elodea*).—The cells of the cortical parenchyma in *Anacharis* are essentially equal in length and arranged in longitudinal files, each file extending the full length of the internode. Each file is derived ontogenetically from a rib-meristem initial which is first recognizable in the 7th to 13th stem unit. The growth of the internode and the ontogeny of a single file of cells in the cortex are thus directly interrelated. The growth curve of the internode follows a sigmoid course, with a prolonged period of acceleration and a relatively brief period of deceleration. The curves for rate of increased in cell size and of cell number are likewise sigmoid and related in a simple fashion to critical features of the growth curve of the organ as a whole. This material illustrates in the simplest concrete form interaction of cell multiplication with cell enlargement during the growth of the organ.

Atchison, Earlene, The Blandy Experimental Farm, University of Virginia, Boyce, Va. *A preliminary phylogenetic study of the Myrtaceae.*—Six genera of the subfamily Leptospermoideae, including *Eucalyptus, Tristamia, Backhousia, Leptospermum* and *Callistemon,* have consistently been found to have a haploid chromosome number of 11. This subfamily is principally confined to the Tasmanian archipelago. Chief variations in chromosome number are found in the subfamily Myrtoideae; four genera, *Myrtus, Eugenia, Feijoa, Psidium,* have shown $2n$ numbers ranging from 14 to ca. 88. The distribution of the Myrtoideae is worldwide in the tropical and subtemperate regions, with the center of specific and generic variation in tropical America. The combined evidence from studies of the geological record, geographical distribution and cytological variation points toward an ancestral Myrtoideae originating in the western hemisphere, spreading worldwide, and giving rise to a branch variation in Southern Asia (Leptospermoideae).

Baldwin, J. T., Jr., The Blandy Experimental Farm, Boyce, Va. *Hevea: A first interpretation.*—*Hevea* is a genus of nine—or fewer—species characterized by much introgressive hybridization (one could with reason reduce the genus to a single species) and, in many cases, by distinct ecotypes and populations. When entities are in contiguous habitats, and especially when the usual conditions are disturbed, hybridization frequently occurs. From the man-modified areas around Manáos and Iquitos, for example, many segregants from interspecific hybridization have been given nomenclatorial notice, and the writer now considers, for instance, that *H. paludosa* and *H. humilior* from Iquitos are probably representatives of a hybrid swarm of *H. confusa* × *H. guianensis* (?). Along the Rio Negro *H. confusa* is typically reddish-fruited: the character is important in interpreting the genus. Because of strong ecological selection, seed or seedlings taken from the wild and cultivated are likely to show more variation than trees in nature: a small population at the Belterra Rubber Plantation established from lower Rio Negro seedlings consists of various segregants of *H. Benthamiana* × *H. Spruceana.* A comparable population was observed, however, on the Rio Trombetas.

The Rio Uaupés area of Brazil contains seven species of *Hevea* and many variants. It is doubtless the center of generic variability. Significant happenings in the history of the genus occurred there. The very closely related *Cunuria* is represented there by three species and exhibits certain characters in common with *Hevea. Micrandra,* another relative, is also of frequent occurrence there. Near the mouth of the Uaupés, on a March afternoon, there were audible to me as I stood in one spot the fruit-explosions and seed-falls of two species of *Hevea,* two of *Cunuria,* and one of *Micrandra.*

H. rigidifolia, collected for the second time, was established at Belém, where under disease-favorable conditions, it appears free from South American leaf blight. *H. pauciflora,* typical of the hills of the upper Rio Negro region, was also established at Belém, where its seedlings grew faster than those of other species. This entity may well be one of the oldest in the genus.

Three approaches to solution of the yield and disease-free problem of rubber for this hemisphere are suggested.

BALL, ERNEST, Harvard University, Cambridge, Mass. *Attempted transplants in the shoot apex.*—Pieces of tissue approximately 200 μ long by 100 μ wide and 100 μ deep were excised from the centers of the shoot apices of *Lupinus albus* L. These explants were (1) exchanged for similar-sized bits of tissue in the shoot apices of other lupine plants, (2) rotated horizontally through 180° and replaced in the original apex, (3) exchanged for similar-sized bits of tissue in the apices of *Coleus Blumei* Benth., *Vicia Faba* L., and *Tropaeolum majus* L. The plants were kept in high humidity. Asepsis was confirmed by placing many of the operated buds in sterile nutrient media. Without exception, the transplants soon shrank in size and died. Grafting appears to be unsuccessful in this region of the plants studied. Prime reason for the immediate death of the transplant was the "isolation" of the latter by the crushed cell walls on the wound surfaces. These masses of debris formed a layer on the outside of the wound surfaces of the stock through which water apparently did not pass to the transplant. A further contributing factor to the failure of these grafts was the peculiar type of callus formed by the cut tissues of the shoot apex. Correlated with the absence of intercellular spaces, the callus which formed beneath the wound surface of the shoot apex resembled cork cambium in the evenness of its outer surfaces; the layer of crushed cell walls over the wound surface was never penetrated by the callus cells. These investigations further demonstrated an essential difference between the embryonic tissues of the shoot apex and the older, subjacent, maturing tissues of the shoot. The latter can easily be grafted, while the former cannot. The older tissues (a short distance beneath the shoot apex) produced finger-like callus from the cut edges of both adjacent parts which penetrated in certain places the layer of crushed cell walls upon the wound surfaces and brought about the histological union of the cells.

In spite of the fact that the transplant, which constituted a large portion of the original shoot apex in each case, had died after the operation, the remaining tissues of the dome of meristem, though small in extent, continued to grow and to exercise their characteristic dominance over the subjacent axillary buds. None of the latter ever sprouted during the course of these experiments. The remaining tissues in the shoot apex on both sides of the excised transplant reconstituted two new shoot apices, each of which produced by its growth a new shoot. This behavior demonstrated that none of the tissues of the shoot apex possessed a specific, irreversible destiny; any of the cells may develop into complete shoot apices.

BEATTY, ALVIN V., University of Alabama, University, Ala. *A continuous flow method for growing penicillium and other fungi.*—A pyrex cylinder, 15 inches high and 4 inches in diameter, fitted with a two-hole 4-inch stopper at the top and a No. 8 two-hole stopper at the bottom, is used as a culture chamber. A cylindrical core of porous firebrick suspended by a wire from the top stopper is used as the substratum. The feed tube is centrally located in the top stopper and comes from the 5-liter nutrient broth flask fitted with a Berkefeld filter for air displacement. The nutrient broth passes from the flask through a drop-counting chamber to the core. The other opening in the top stopper is connected with a Berkefeld filter which is used as a breather for the culture chamber. One of the openings in the bottom stopper is connected to the collecting bottles and the other to a suction pump which allows a continuous, sterile gas exchange within the culture chamber. The sterile core is inoculated with a spore suspension by means of a hypodermic needle inserted in the rubber tubing of one of the top openings. The culture medium is adjusted to pH 7 and the nutrient flasks are changed under sterile conditions produced by means of an ultraviolet lamp. Once a pure culture is produced with the aid of the autoclave and the ultraviolet lamp for sterilization, a continuous growth of mold is maintained.

BLASER, H. WESTON, Cornell University, Ithaca, N. Y. *Variability in the morphology of some angiosperm shoot apices.*—Structural differences in shoot apices have been described between genera, species, and the several branches of individual plants. Investigations were undertaken in an effort to correlate some of these differences. Examination of apices of many plants has begun and there is evidence that these differences are not morphologically significant.

The apical region of the stem of *Stellaria aquatica* L. is variable in form from a nearly flat apex with marginal erect leaf primordia to an elongate conical apex with lateral horizontal leaf primordia. The "tunica" varies from two to four (1–5?) layers in thickness and its definition is in no case clearly marked. Leaf primordia arise from at least three cell layers.

Bryophyllum pinnatum Kurz., in contrast, shows uniformly flat, small, shallow meristems. Erect leaf primordia arise marginally at the apex. Any effort to denote zones or areas is limited by the small size of the apex and the small number of cells in noticeably meristematic condition. The "tunica" is perhaps biseriate and initiates the formation of leaf primordia.

BLOCH, ROBERT, Osborn Botanical Laboratory, Yale University, New Haven, Conn. *The idioblastic development of the trichosclereids in the air root of Monstera deliciosa.*—In the air root long, pointed sclereids occur ("trichosclereids") which grow intrusively between the cortex cells along the course of the developing intercellular space system. The trichosclereids, unlike the brachysclereids of the hypodermal sclerenchyma, are the result of (1) early differential, polar divisions of cortex cells; (2) long-continued proliferation of the embryonic

initials thus formed; (3) subsequent sclerosis. The distribution of these "idioblasts" can be traced back in the fundamental tissue to a pattern of differential cell divisions, occurring at the basal (proximal) ends of cell files in the rib meristem; their development is the outcome of specific differentiation rather than of uncontrolled growth of solitary cells.

BOND, LORA, Wellesley College, Wellesley, Mass. *Responses of pea roots to the application of certain growth substances.*—Characteristic changes in certain tissues follow the application of some growth substances in lanolin to roots of *Pisum sativum* seedlings. The application of asparagine, indoleacetic acid, or 2, 3, 5-triiodobenzoic acid has little effect. Changes are very marked following the treatment of uncut roots with tryptophane or with 2, 4, 5-trichlorophenoxyacetic acid, or of cut roots with the latter substance. Following application of tryptophane, an increase in the diameter of the root is brought about by proliferation chiefly of the cambium and xylem parenchyma. There are some divisions in the pericycle and a few in the endodermis. Scattered cells in the stelar region mature as scalariform tracheids. The following effects are visible in pea roots treated with 2, 4, 5-trichlorophenoxyacetic acid: (a) an increase in the diameter of the root, especially near the tip, brought about by an increase in the number of cells with no marked enlargement of cells; (b) stretching and tearing of cortical parenchyma cells in the enlarged part of the root; (c) hypertrophy of epidermal cells; (d) an increase in the number of root hairs; and (e) cessation of maturation of vascular tissues. There is active division of the cortical parenchyma cells near the meristematic region of the root, and some proliferation of endodermal cells is evident, but most of the meristematic activity is in the pericycle region. The pericyclic proliferation occurs in a complete ring near the root tip; at higher levels of the root it appears largely opposite the xylem arms and is limited in the mature region to the vicinity of young branch roots.

CLAUS, EDWARD P., University of Illinois, College of Pharmacy. *A study of the anemophilous plants of Puerto Rico.*—A survey of the flora of Puerto Rico has disclosed the existence of certain anemophilous plants which may be of potential danger to allergic individuals. Tropical, subtropical, and temperate species are represented in this flora. Some of the wind-pollinated species growing in Puerto Rico are identical with those of North, Central and South America. It has been determined in the latter locations that many of these plants cause allergy. However, in Puerto Rico there have been no clinical studies of wind-pollinated plants either naturalized or endemic. Pollen counts are now being determined. This paper suggests as a basis for future clinical study the relationship between the uninvestigated anemophilous plants of Puerto Rico and the allergenic plants of North, Central, and South America.

CORNMAN, IVOR, Army Service Forces, Washington, D. C. *Alteration of mitosis by coumarin and parasorbic acid.*—Two chemically related compounds occurring naturally in plants inhibit germination and growth. Parasorbic acid is 2-hexene-1, 5-olide and coumarin contains the same lactone ring with a benzene ring added. In onion and lily roots, a saturated aqueous solution of coumarin blocks all mitoses within 2 to 3 hours, producing the initial phenomena of a colchicine effect: destruction of the spindle, with the resultant interruption of anaphases, and accumulation of metaphases in which the chromosomes shorten and split, but centromere division is delayed. These interrupted mitoses form tetraploid nuclei, or binucleate cells in some of which there is a bridge between the nuclei. Coumarin also prevents the entry of cells into mitosis, and as a result, mitoses gradually disappear and the polyploidizing cycle is not repeated. Parasorbic acid in saturated solution causes an accumulation of onion root metaphases, but apparently only by slowing mitosis. These retarded metaphases eventually continue in normal anaphases and telophases. Mitotic abnormalities with parasorbic acid are rare. Inception of mitosis is also prevented by parasorbic acid. Sorbic acid, the straight-chain isomere of parasorbic, is highly toxic. Cytological data on dicoumarol, a related compound also occurring in nature, are not yet available, but it too is toxic in saturated solution.

CUTTER, VICTOR M., JR., Department of Botany, University of Minnesota, Minneapolis, Minn. *Observations on the chromosomal morphology of Neurospora tetrasperma with the aceto-carmine smear technique.*—Recent studies by McClintock and others indicate that it may be possible in the fungi, as in other organisms, to correlate genetic behavior with specific chromosomal morphology. Previously it was generally assumed that in most cases the minute nature of the fungus nucleus and its chromosomes precluded the effective use of cytological procedures in genetic analysis. The existing controversy concerning the chromosome number and the nuclear behavior during meiosis in *Neurospora* may be due to inherent differences in the strains investigated, or may reflect in part differences in the investigational techniques employed by various workers. These divergent interpretations accentuate the need of a correlated study of a number of strains in this genus by means of a uniform staining procedure.

The present investigation explores the practicability of utilizing a rapid smear technique in the study of these organisms, and presents observations on the mitotic and meiotic divisions throughout the life cycle of a strain of *Neurospora tetrasperma* Shear & Dodge. In general the nuclear behavior of this material corresponds to that described for other strains and species of *Neurospora*, but minor differences occur. Several useful modifications of the standard aceto-carmine smear technique are discussed.

DAHL, A. ORVILLE, University of Minnesota, Minneapolis, Minn. *Pollen morphology in the Commelinaceae Reichenb.*—The pollen grains of *Commelinantia anomala* (Torr.) Tharp are distinctive in possessing three germinal furrows rather than the single furrow or pore found in *Tradescantia* L., *Commelina* L. and other members of the family as well as in most of the Monocotyledons that have been investigated. In *Commelinantia Pringlei* (S. Wats.) Tharp, a species whose taxonomic position has been somewhat problematical, the pollen grains are more or less transitional between those of *C. anomala* and *Tradescantia.*

DOTY, MAXWELL S., Northwestern University, Evanston, Ill. *Critical tide factors in the vertical distribution of marine organisms.*—Investigation of the tidal phenomena illustrated by a marigram of the tidal variations occurring during the year at San Francisco reveals levels at which there are at least 2- or 3-fold abrupt increases or decreases in the length of exposure to the various physical environmental factors. Along the west coast of the United States the littoral environmental factors vary quantitatively but only the tide range and these tide factors are in correlation, respectively, with the overall vertical distribution of intertidal organisms and with the breaks between the floral and faunal zones throughout the geographic area surveyed. The life cycle of the algae and animals studied in correlation with the annual variation in the levels at which these tidal factors occur is, seemingly, the fundamental avenue of approach to an understanding of the vertical distribution of the littoral marine organisms.

EIGSTI, O. J., Department of Botany, Northwestern University, Evanston, Ill. *Colchicine—A bacterial habitat.*—Cultures of microorganisms growing in a 1 per cent aqueous solution of colchicine have been observed in the laboratory at different intervals during the past five years. Coincidentally bacterial colonies in sucrose-agar-colchicine cultures of pollen tubes were noted. Isolation by the usual bacteriological methods gave pure cultures of a gram negative, motile, non-spore forming, bacillus-type. This isolation was made from a nine-months old stock solution of colchicine. In light of the mitotic disturbances and the toxicity of colchicine to many plant and animal cells, such information about colchicine as a habitat for bacteria is of interest. The thriving growth without complete nutrient media suggests some problems of metabolic significance. Specific identification and certain phases of metabolism of the culture in relation to colchicine are being investigated currently.

FARR, WANDA K., AND SOPHIA H. ECKERSON, Celanese Corporation of America, Cumberland, Md., and Cleveland Heights, Cleveland, Ohio. *The conidiophore of Aspergillus niger.*—Studies of developing conidiophores reveal the complex physical and chemical nature of their cell walls and indicate the function of these combined factors in determining the properties of the mature conidiophore.

FLINT, LEWIS H., AND CHARLES F. MORELAND, Department of Botany, Louisiana State University, Baton Rouge, La. *Antibiosis in the bluegreen algae.*—During laboratory work with bluegreen algae evidence of algae exudates lethal to other algae was obtained. One of these algae was selected for further studies which have included the cultivation of the alga in the laboratory, the production of the antibiotic exudate under laboratory conditions and some characteristics of the exudate, including its relation to root growth and to bacteria. The results of these studies as described in this paper appear to indicate another source of antibiotics, of possible significance in relation to plant nutrition or pathology.

GAST, P. R., Harvard University, Cambridge, Mass. *Sterile culture of aspen roots on cuttings and as excised organs.*—Aspen roots cultured on cuttings do not thrive in sterile media. Supplemented with organism, especially a pink yeast resembling *Torulopsis pulcherrima,* much more thrifty root growth is found. Such roots grown as excised organs thrive better. No combination of accessory growth substances has been found which will admit of unlimited growth and sub-culturing. Among many hundreds with initial length of 15 mm., 59 grew to lengths of 25 to 180 mm. before ceasing growth. The longer grew as much as forty days before the sudden development of the pink yeast which apparently persisted as an endophyte. The longest root grew to 370 mm. in 104 days after accidental infection with *Alternaria sp.* and *Cladasporium sp.* but the result could not be duplicated with controlled inoculation. Heterotrophism in the aspen root is presumptive.

Sterilization of cuttings utilizes immersion in 0.004 M $NH_4H_2PO_4$ and 50 p.p.m. Cl from chlorox for 48 hours, followed by 0.2 per cent bromine solution for 8 minutes. Sterilization of excised roots utilizes immersion with constant turning in 250 p.p.m. succiniodimide for 30 minutes during exposure to 300 microwatts per sq. cm. of ultra violet of 2537 Å. An apparatus for growing plants with roots in sterile media and with shoots exposed to the atmosphere is described.

GATES, FRANK C., Kansas State College, Manhattan, Kan. *Moss in the revegetation of an area in Northern Michigan.*—A series of detailed plottings of the occurrence of plants revegetating a 38 sq. meter reserve on a sand road intersection, following removal of a building which had occupied the site for six years, made it possible to record the entrance and rapidity of spread of the moss, *Ceratodon purpureus.* Moss appeared in the 5th year on 1 sq. meter out of 38, making but very little increase in the drouthy 30's but rapidly in the wet seasons following, namely, from 6 sq. m. in 1939 to 34 sq. m. in 1945.

GILES, NORMAN H., JR., Osborn Botanical Laboratory, Yale University, New Haven, Conn. *Induced biochemical mutants in Absidia glauca.*—By utilizing a procedure similar to that originated by

Beadle and Tatum for the ascomycete *Neurospora*, biochemical mutants have been obtained in the heterothallic phycomycete *Absidia glauca*. The general technique was as follows: (a) suspensions of sporangiospores were irradiated with ultraviolet; (b) spores were dispersed into "complete" medium (enriched malt-yeast extract agar) in Petri dishes and colonies derived from single spores were isolated individually onto agar slants of the "complete" medium; (c) inoculations from these cultures were made into a "minimal" medium (glucose, asparagine, salts). Lack of growth in the minimal medium indicates a mutation involving the loss of synthetic capacity for some diffusible substance essential for growth. Of the several such mutants obtained in this manner, the requirements of four have been identified to date. Single substances added to the "minimal" medium are required for growth in each instance, as follows: pantothenic acid (mutant #196); *p*-aminobenzoic acid (#1200); histidine (#10 and #50—independent mutations). The growth of these mutants, as measured by the dry weight of mycelia, is a function of the concentration of the required substance. The pantothenicless mutant is unable to grow on a medium containing a mixture of B-alanine and pantoyllactone. As far as the present evidence indicates, this mutant thus appears to be biochemically and genetically homologous with the two pantothenicless mutants reported in *Neurospora crassa*, which also are unable to complete the synthesis of pantothenic acid from its two immediate precursors. Heterocaryon formation, followed by the reisolation of both component strains, has been achieved with certain of the mutants. To date direct studies of the inheritance of the mutant characters have been unsuccessful due to difficulties in securing zygospore germination.

Hoskins, J. H., and A. T. Cross, University of Cincinnati, Cincinnati, Ohio, and University of Notre Dame, Notre Dame, Ind. *On the occurrence of non-functional megaspores and pollen in a new species of Pachytesta.*—A new species of the Pteridosperm seed *Pachytesta* from the Des Moines Series of Iowa contains in the area between the functional megaspore membrane and the upper limits of the nucellus, a number of non-functional megaspores and pollen-grains. The majority of the non-functional megaspores occur in tetrahedral groups. The size of the spores and their relationship to the structure, organization and preservation of the micropylar area offer no evidence favoring the external origin of these spores. On the other hand their position, occurrence in tetrads, and histological similarity to the functional megaspore indicate *in situ* origin and development.

Found associated with the megaspores were four small, trilete microspores, and approximately 40 pollen grains of one kind, referable to the genus *Florinites*. The numerous references to pollen in seeds of this genus prove to be founded on the material originally figured and described by Brongniart. On the basis of the descriptions and illustrations of this pollen by Brongniart and Renault it is not impossible that it, too, is referable to *Florinites*. No comparison can be made with Grand-'Eury's unillustrated report of multi-cellular pollen grains in *Trigonocarpus pusillus*. The uniformity of type, which opposes the thesis of "foreign pollen," and some evidence of a similar type of pollen in seeds of this group previously studied, indicate the possibility that these grains are the true pollen of this seed, in spite of the fact that pollen of the *Florinites* type has already been described for genera of both the Cordaitales and Coniferales.

Johnson, Marion A., Rutgers University, New Brunswick, N. J. *Development of the tomato embryo.*—The Marglobe variety of *Lycopersicon esculentum* Mill. was used in this study. The early proembryo consists of four cells arranged in the form of a linear tetrad. The terminal cell is usually the first to divide and produces the cotyledonary part of the embryo. The hypocotyl, dermatogen, periblem and plerome of the root originate from the subterminal cell while the one beneath it gives rise to the root primordium and distal part of the suspensor. The basal cell which is attached to the inner integument contributes the remainder of the suspensor. Growth from the terminal and subterminal cell establishes the apical part of the proembryo as a sphere and later as a cylinder. Vacuolation in the spherical stage blocks out the cortex and pith with the intervening cells remaining as the forerunners of the vascular system. Elongated procambial cells appear at several levels in the hypocotyl and extend acropetally into the cotyledons and root. The cotyledons are elevated to a height of about .8 mm. by terminal growth after which they are further lengthened by a rib meristem and broadened through the activity of a row of sub-marginal initials. In mature seeds the shoot apex is a dome-shaped mass of meristematic tissue consisting of a tunica of two or three cell layers clothing the corpus which gives rise to long files of pith cells.

Karling, John S., Columbia University, New York City. *Keratinophilic chytrids.—Rhizophydium keratinophilum* n. sp. and *Perirhiza endogena* n. gen. et n. sp. are new species which have been isolated and grown on keratinized substrata such as human hair, skin, finger and toe nails, feathers, wool, hoofs, horns, snake skin, etc. They are thus keratinophilic in their growth requirements and appear to derive most of their energy from cystine, a *di* (*b-thio-a-amino propionic acid*) or sulphur-containing body obtained as a decomposition product of keratinized tissues.

Kosanke, R. M., and J. T. Buchholz, Illinois Geological Survey and University of Illinois, Urbana, Ill. *Pollen grains of the genus Podocarpus.*—The species of *Podocarpus* are numerous, and there exists considerable variation in size and form of the vegetative organs. With this in mind, it was decided to examine the pollen grains of as many

species as were available to determine the pollen morphology, and to explore the possibilities of identification of species of *Podocarpus* by pollen grains.

A total of 44 samples were collected from a number of herbaria which included 34 different species from five of the six sections of the genus. Section *Microcarpus* has but one species, and no pollen was available for study.

All of the pollen grains examined were either two- or three-winged. Species examined from the section *Dacrycarpus* were all three-winged. The section *Nageia* has been divided by Florin into two groups based on leaf position, stomata distribution and the presence or absence of a fleshy receptacle. *Podocarpus Wallichianus,* in one of the two groups, has three-winged pollen while *P. nagi* in the other group has two-winged pollen. The sections *Stachycarpus* and *Eupodocarpus* containing a large number of species have all had two-winged pollen. Pollen from the section *Polypodiopsis* (*P. vitiensis*) was immature but probably was two-winged. The largest pollen encountered was that of *P. ferrugineus* of the section *Stachycarpus*. The pollen grains consistently measured over 80 microns including the wings while the body of the grain usually averaged over 65 microns in width.

La Rue, Carl D., and Fern Reissig, University of Michigan, Ann Arbor, Mich. *Grafting in leaves.*—Leaves of *Kalanchoe rotundifolia* graft readily; either attached to plants or detached. Grafts have been made between proximal and distal halves of leaves; between right and left halves; between two leaf tips; between two leaf bases, etc.

Leaf grafts have been made also in *Kalanchoe tubiflorae, Cotyledon orbiculata, Byrnessia Weinbergii, Echeveria pulyinata,* and other succulents.

Leaves of *Kalanchoe rotundifolia* and *K. tubiflorae* have been intergrafted.

V-shaped cuts have given best results, although cross, and diagonal cuts also have succeeded.

Thin-leaved plants are difficult to graft by abutting scion on stock, but *Zebrina pendula* leaves were thus grafted. A better method consists in peeling the epidermis from each leaf and pressing together the exposed mesophylls. This technique was successful with *Saxifraga sarmentosa.* Stems of *Kalanchoe rotundifolia* have been grafted into leaves of *K. rotundifolia, K. tubiflorae, Crassula arborea, Cotyledon orbiculata* and others.

In leaf grafts, union is made by callus which develops on the cut surfaces, especially at the cut ends of bundles. If contact between stock and scion is poor, cork development may partially or completely prevent union.

Stems grafted into leaves unite with the vascular bundle of the mid-rib of the leaf.

McDonough, E. S., Marquette University, Milwaukee, Wis. *The cytology of Sclerospora macrospora (Sacc.) in relation to its taxonomic position.*—A comparison of the cytological features of the fungus herein referred to as *Sclerospora macrospora* with the published reports of both amphigynous and paragynous Phytophthoras casts doubt on the advisability of including the parasite in the genus *Phytophthora* as was done by Tanaka. From 6 to 9 nuclei were observed in the young antheridium and from 30 to 45 nuclei in the oogonial initial. Simultaneous mitoses were found in both sex organs. Several nuclei were found to enter the oosphere, one single male nucleus uniting with a solitary female pronucleus, the rest disintegrating. It has long been considered that the number of nuclei present in the mature oospore is a good criterion of the taxonomic position of a member of the Peronosporales, since all members of the Albuginaceae studied cytologically have coenocytic resting spores while *Sclerospora* (as exemplified by *S. graminicola*), *Plasmopara, Peronspora,* and *Phytophthora* have a delayed union of the pronuclei, the mature oospore being monocaryon. The development of the oospore of *S. macrospora* seems to be similar to that of *Albugo tragopogonis.* However, there would seem to be little justification for placing *S. macrospora* in the genus *Albugo* on the basis of the asexual phase. Further work may well lead to the erection of a new genus.

Matzke, Edwin B., Columbia University, New York, N. Y. *The role of surface forces in three-dimensional cell shape determination.*—Data have been assembled on the three-dimensional shapes of bubbles in specially constructed uniform and variant foams. These results were then compared with similar data available on lead shot compressed in a steel cylinder to eliminate interstices, and with the published data of various authors on the three-dimensional shapes of cells in tissues. Such comparisons show that in average number of contacts, in number of pentagonal, triangular, heptagonal and octagonal faces, in number of combinations of faces, and in number of tetrahedral angles, the cells were intermediate between bubbles in foam and compressed shot. In certain respects, such as in the number of contacts, the cells showed closer similarity to the bubbles than to the compressed shot. Since surface forces are of considerable importance in the soap films, and of much less relative significance in the compressed lead shot, the conclusion is drawn that surface forces play a part—not necessarily the most important part—in the determination of three-dimensional cell shapes at or near the plant meristems.

Melhus, I. E., G. Semeniuk, J. R. Wallin, G. M. Watkins, and G. J. Goodman, Iowa Agricultural Experiment Station, Ames, Iowa. *Comparative development of some United States, Mexican and Central American corns at different latitudes and altitudes.*—Corns of different geographic origin were grown at nine stations in the United States, Mexico and Guatemala at latitudes from 14° to 42° and altitudes from near sea-level to 8,000 feet. These corns included inbreds, single- and double-crosses from the United States and open-pollinated populations from all three coun-

tries. Latitude and altitude influenced the development of the United States and Valley of Mexico corns more than the highland Guatemalan corns. The vegetative and floral characters of the highland Guatemalan corns remained relatively constant. The United States corns were early and varied alike in development. Their maturity ranged from 75 days at Weslaco, Tex., with an altitude of about 100 feet, to 155 days at Quetzaltenango, Guatemala, at 8,000 feet. The Valley of Mexico and Guatemalan corns were of definitely longer season than the United States corns. The Guatemalan corns were generally strong in root and vegetative development and in the production of multiple ear shoots and profusely branched tassels. The apparent stability of certain vegetative and floral characters suggests that there are only a few major groups in *Zea mays* and that within these groups there are many minor variants.

MEYER, SAMUEL L., Emory University, Ga. *Some aquatic plants of importance in the breeding of Anopheles albimanus in Panama.*—The important vector of malaria in Panama, as in the entire Caribbean region, is *Anopheles albimanus.* It is a common species in the lowlands and breeds in a variety of habitats, including fresh to brackish water, but most abundantly in full sunlight in bodies of water where there are mats of floating vegetation. The most important elements in the composition of the vegetation mats in the Gatun and Chagres Rivers are: *Naias* sp., *Eichornia azurea, E. crassipes, Cabomba aquatica, Jussiaea natans, Salvinia natans, Azolla* sp., and *Pistia stratiotes.* Of these, undoubtedly the most important, from the standpoint of the breeding of *Anopheles albimanus,* is *Naias* sp.

MURPHY, JOHN B., De Paul University, Chicago, Ill. *Embryo-sac development of Allium cernuum Roth.*—Since Bambacioni discovered that *Lilium* belonged to the *Fritillaria*-type of embryo-sac instead of the traditional *Lilium-type,* much research has been done on *Lilium* and other genera of the *Liliaceae.* This paper is concerned with a comparative study of the embryo-sac development and embryo-sac type of a wild species of *Allium.*

The megasporangium of *Allium cernuum* Roth follows the usual *Lilium* development. Before the style is very far developed a single archesporial cell is differentiated. As the archesporial cell begins its first meiotic prophase the inner integument appears as a fold at the base of the ovule. The outer integument differentiates about the time that the archesporium is in synapsis. The integument grows with greater rapidity than the nucellar tissue and completely envelopes the ovule before meiosis is completed.

The archesporium differentiates about three or four days before the flowers emerge from the bud. The heterotypic division results in two dyads. The micropylar dyad disintegrates without further nuclear division. The embryo-sac is thus formed from the chalazal dyad. The chalazal dyad undergoes three divisions resulting in an eight-nucleate sac. The mature embryo-sac consists of a large vacuolated egg, two smaller synergids, one of which disintegrates before fertilization, two polar nuclei and three antipodal cells which frequently do not persist.

The embryo sac of *Allium cernuum* was found to be of the usual *Allium* type with no indications of abnormality. The megagametophyte is mature about six to twelve hours after the opening of the flower and fertilization apparently occurs within three days after the megagametophyte is mature.

NEWCOMER, EARL H., University of North Carolina, Chapel Hill, N. C. *The duality of mitochondria in plants.*—This study is largely concerned with the validity of Guilliermond's and Bowen's theories and evidence of the dual nature of mitochondria in fixed materials of plants. The problem of cytological technics relative to the demonstration of mitochondria is also considered and some suggestions are proffered for a simpler and more rational terminology in reference to mitochondria.

A total of one hundred eighty-nine mitochondrial technics, including all those found in the literature, was used in the study. None were found to give an adequate nor consistent preservation of the mitochondria throughout a tissue. Some success was experienced in attempting to produce a complete fixation, i.e., the combination of the heretofore mutually exclusive acid and basic fixation images.

The theory of the duality of the mitochondria in plants promulgated by Guilliermond is shown to be without adequate cytological support and the *a priori* assumption upon which it is based seems invalid.

Similarly, Bowen's claims to have distinguished two categories of mitochondria-like elements in the cytoplasm of plant cells, the plastidome and pseudochondriome, based upon differences in function, morphology, staining behavior and behavior during cell division, are not supported by the results of this study.

The evidence from this study supports the suggestion that the functions of mitochondria are multiple, variable, or perhaps eclectic in nature, subject to the genetic and metabolic requirements of the organism.

Further research and morphological studies with the technics available at the present time seem futile. The subject needs a new approach and it is believed that the emphasis should be primarily upon the securing of an adequate technic which will yield comparable results in the hands of any competent investigator.

OVERBEEK, J. VAN, Institute of Tropical Agriculture, Mayagüez, Puerto Rico. *An alga as a beach former in the tropics.*—*Halimeda Opuntia* (*Linnaeus*) Lamouroux is a calcified green alga growing in great abundance on coral reefs in shallow water. It was found that the sand of the beaches of coral islands on the west and south coasts of Puerto Rico

was often 90 per cent composed of the remnants of this alga.

OVERBEEK, J. VAN, Institute of Tropical Agriculture, Mayagüez, Puerto Rico. *Colored slides of tropical crops and plants.*

PADDOCK, ELTON F., Ohio State University, Columbus, Ohio. *A theoretical advantage of using autotetraploids in crop plant breeding.*—In crop plants, chromosome pairing is usually undisturbed in meiosis of intervarietal hybrids. If, for example, there are 12 pairs, 61.23 per cent of the individuals in the first backcross generation will theoretically have received 6 or fewer chromosomes from the undesirable parent. Among these, 1/12 will possess the single desired chromosome being introduced from the otherwise undesirable parent. They may be identified at once if the characteristic being introduced is due to a dominant genetic factor. This 1/12 alone, or *all* the first backcross individuals may be again backcrossed. In either case, 12.59 per cent of the individuals in the second backcross generation will theoretically have received exactly 1 chromosome from the undesirable parent.

If, instead, an autotetraploid of the standard variety to be improved had been used in the initial cross, and the diploid form used for the backcrosses: 93.36 per cent of the individuals in the first backcross generation would have received 6 or fewer chromosomes; 26.81 per cent of the individuals in the second backcross generation would have received exactly 1 chromosome from the undesirable parent. Thus, there is more than twice as good a theoretical chance to achieve unencumbered transfer of a single chromosome within two backcrosses. This speed greatly reduces the opportunity for crossing over to intermix undesirable chromosome segments into the standard complement. The above calculated percentages are based on the assumption of no crossing over hence are only approximations for the first and second backcrosses, and would be even less meaningful if calculated for a third or later backcross.

PHILLIPS, H. M., Emory University, Ga. *The formation of nucleoli in Erythronium.*—Nucleoli in plants arise from nucleolar organizers and nucleolar bodies associated with Sat-chromosomes or chromosomes with secondary constrictions. Usually the numbers, sizes and exact points of origin of nucleoli are characteristic of the species.

Species of *Erythronium* exhibit a peculiar type of nucleolar formation during meiotic and microspore divisions. Nucleoli begin their formation during telophase; they continue their development throughout the early prophase of the following division. As a result of this type of formation, accompanied by constant fusing, it is practically impossible to determine accurately the "basic" number of nucleoli.

From thirty to fifty dark, chromatic bodies, scattered throughout the nucleus, are associated with the chromosomes. Many of these "dots" definitely take part in the formation of the nucleoli. Three to seven of these, each surrounded by a clear, nonstaining area, are always associated with the developing, almost non-chromatic, nucleolus. By midprophase there are usually two to six nucleoli in each nucleus. Each nucleolus has one or more chromosomes attached to it. Often by late prophase only one nucleolus, formed by fusion of several, is present, and it may have the entire set of chromosomes ($n = 12$) attached to it. All attachments are terminal. Only one pair of Sat-chromosomes has been observed in any species studied.

PHILLIPS, H. M., AND CAROLYN WOOD FORMAN, Emory University, Ga. *Desynapsis in Erythronium californicum Purd.*—Slides prepared from three hundred corms of *Erythronium californicum* were studied. Approximately ninety per cent of the meiotic divisions on these slides were irregular or abnormal. Failure of the chromosomes to remain paired at zygotene, or in certain cases failure of the chromosomes to pair, was responsible for this high percentage of irregularity.

In the cells in which desynapsis occurred, univalents were formed. Occasionally cells were found with both univalents and bivalents present. The nuclei of cells possessing only univalents divided mitotically with twenty-four chromosomes migrating to each pole at anaphase. Daughter nuclei were formed. Generally there was no spindle formation for "metaphase II"; the nuclear membranes disappeared at prophase and forty-eight chromatids were found scattered throughout the "cell." Groups of chromatids separated as units within the cell, forming irregular tetrads.

Of the many flower buds that were examined for pollen grain divisions, only a few possessed normal microspores. Practically all irregular tetrads disintegrated. A few microspores were found, however, with eleven, eighteen and twenty-four pairs of chromosomes ($n = 12$).

PHINNEY, BERNARD O., University of Minnesota, Minneapolis, Minn. *Cell length in the parenchyma of the midrib of normal and dwarf-1 maize at various stages of development.*—The expression of the dwarf-1 gene in *Zea mays L.* on leaf development was studied by comparing cell lengths of the parenchyma of the midrib throughout the length of the leaf at various stages of development in normal and dwarf-1 plants. In *very young* leaves these parenchyma cells are more or less uniform in length. As development proceeds they are transformed through differential rates of growth into alternating regions of short and long cells, the longest cells occurring in the ligular region and the central portion of the blade. The developmental pattern then changes in the various parts of the leaf so that *at maturity* the cells in the ligular region are relatively *short* with respect to adjacent areas, maximum cell length being found in the central portion of the sheath and basal third of the blade. At the tip and at the base of the leaf, cell length remains relatively short throughout development. A similar developmental pattern holds for dwarf-1 leaves, except that the

absolute length of the cells throughout the leaf was found to be less than for corresponding portions of normal leaves.

RAPER, KENNETH B., AND DOROTHY F. ALEXANDER, Fermentation Division, Northern Regional Research Laboratory, Peoria, Ill. *Production of penicillin X in submerged culture.*—Members of the *P. notatum-chrysogenum* group are known to produce at least four different natural penicillins (F, G, X, and K). Two or more of these may occur in the same culture broth, the proportions being influenced by the strain used and the cultural conditions employed. In addition to known differences in chemical characteristics and in their inhibitory effect upon susceptible bacteria, some clinical evidence is now available showing unique therapeutic possibilities in the clinical application of one of these penicillins, namely, chloroform-insoluble penicillin X.

Known good "submerged strains" were tested in shaken flask cultures, in rotary drums, and in vat fermenters for their capacity to produce this penicillin. The amount of penicillin X was determined by means of differential assays of chloroform extracts of the culture broth. One of the strains tested, *P. chrysogenum* NRRL 1984.A, was found to produce penicillin X amounting to 15 per cent of the total yield. Unsuccessful attempts were made to isolate an improved penicillin X-producing strain from among obvious variants occurring in streak plates of this culture.

Conidia of this same strain were then exposed to ultraviolet radiation, plated out, and random isolations made. One of these isolates, designated NRRL 1984.N22, when tested in shaken-flask culture in repeated experiments, produced from 100–125 u/ml of penicillin of which 50–65 u/ml represented penicillin X. Later tests in a 600 liter vat fermenter showed total yields of approximately 200 u/ml, 50 per cent of which assayed as penicillin X. By weight, this represented approximately 67 per cent of the total penicillin.

Culturally the two strains, NRRL 1984.N22 and its parent NRRL 1984.A, are strikingly similar.

ROBBINS, WILLIAM J., AND ILDA MCVEIGH, Department of Botany, Columbia University, and The New York Botanical Garden, New York, N. Y. *The effect of l-hydroxyproline on trichophyton mentagrophytes.*—The growth of *T. mentagrophytes* on a basal salts medium to which asparagine or any one of 14 amino acids has been added as the nitrogen source was inhibited by l-hydroxyproline. Concentrations of 1 to 8000 almost completely inhibited its growth. Two pleomorphic forms were found to respond in a somewhat similar manner while a third form was resistant. The effect of hydroxyproline was antagonized by l-proline but not by 13 other amino acids. Hydroxyproline was found to be injurious to four other dermatophytes investigated. These included representatives of three genera. It did not inhibit the growth of 19 fungi including species of *Phycomyces, Rhizopus, Aspergillus, Penicillium, Rhodotorula, Agaricus, Stereum, Polyporus, Fomes, Ceratostomella,* and *Neurospora.*

ROSEN, H. R., AND H. W. LARSH, University of Arkansas, Fayetteville, Ark., and University of Oklahoma, Norman, Okla. *Blight of oats caused by an Helminthosporium differing from the one producing leaf spot.*—A destructive disease of oats caused by a species of *Helminthosporium* has been found in Arkansas which produces seedling blight, a blighting of leaf sheaths and of whole tillers of various aged plants and a spotting of blades. The disease and the fungus differ markedly from the well known leaf spot (leaf blotch) caused by *H. avenae.* The disease is especially destructive to a number of crown rust resistant and smut resistant varieties, with indications that increased acreages of these varieties may result in increased prevalence of *Helminthosporium* blight. Seed treatment with New Improved Ceresan is either only partly effective or ineffective as a control. There are indications of varietal differences in susceptibility. Control in 1945 was obtained by late fall planting, which resulted in considerable suppression of the disease and in greatly increased grain yields.

SASS, JOHN E., Iowa State College, Ames, Iowa. *Development of endosperm and antipodal tissue in "Argentine waxy" maize.*—"Argentine waxy" maize is a favorable subject for the study of the development of endosperm in relation to antipodal tissue. Three to four days after pollination the endosperm, at first free-nuclear, becomes cellular. As the endosperm enlarges and advances through the nucellus toward the stylar end, the small mass of antipodal tissue is pushed toward the style. Very limited cell division occurs in the antipodal cells. By the tenth day, the endosperm has completely replaced the nucellus, and the antipodal cells are crushed against the stylar end of the kernel. The endosperm at the stylar end becomes lobed and involuted by locally unequal growth. Usually one prominent lobe develops, becomes packed with starch and develops a typical aleurone layer. A marginal section of the lobe has the appearance of an island enclosed in a surface layer, and could be mistaken for enlarged antipodal tissue. Serial sections during kernel development to maturity show that the lobe is continuous with the other portion of the endosperm. The starch in the lobe and elsewhere in the endosperm gives the same iodine reaction. Clarification of the history of antipodal and endosperm tissue in diverse types of maize is essential for studies in which starch and other cell contents may differ in monoploid and triploid cells.

THOMPSON, R. H., Department of Research and Education, Solomons Island, Md. *Immobile Dinophyceae of Maryland.*—In the summer of 1942 a number of immobile or alga-like Dinophyceae were found, none of which have been previously reported from the western hemisphere. Seven of these are being reported upon, of which one is a new species. They are: *Gloeodinium montanum* Klebs, *Cysto-*

dinium Steinii Klebs, *Cystodinium Bataviense* Klebs, *Hypnodinium sphaericum* Klebs, *Tetradinium Javanicum* Klebs, *Stylodinium globosum* Klebs, and *Stylodinium longipes* sp. nov.

TURRELL, F. M., University of California, Citrus Experiment Station, Riverside, Calif. *Estimating fruit surfaces.*—Volumes of spherical or nearly spherical fruits are easy to calculate or measure, and surfaces of spherical fruits are easy to calculate but surfaces of non spherical fruits are very difficult and laborious to measure or calculate. Tables have been prepared from which both surfaces and volumes may be obtained by using simply the measurements of the polar axes and equatorial axes, when the fruit are oblate spheroids (spheres flattened at poles) and prolate spheroids (spheres extended at poles). The tables may be employed for bodies of any size and ranging in shape from spheres to cookies or from spheres to tooth picks.

WARMKE, H. E., Carnegie Institution, Cold Spring Harbor, N. Y., and Institute of Tropical Agriculture, Mayagüez, Puerto Rico. *A study of spontaneous breakage of the Y chromosome in Melandrium.*—The Y chromosome has been found to undergo spontaneous breakage, ranging as high as 5 per cent in 2A XXY stocks of *Melandrium*. Plants of this constitution may give rise to offspring with deficiencies of either the differential arm of the Y or the homologous arm, or both differential and homologous arms. These deficiencies appear to be terminal and are of varying lengths. Breakage of the differential arm of the Y is greater in plants which have previously suffered loss of part or most of the homologous arm, and cytological examination has shown that deficient Y chromosomes are constant in size in all parts of an affected plant. The above facts suggest that breakage of the Y chromosome is of meiotic origin; it probably is not due to resolution of the usual heterozygous inversion bridge, however, for the following reasons: (1) Breakage occurs in selfs of highly inbred stocks where heterozygosity is not expected. (2) The differential arm of the Y has no homologue and does not synapse, yet suffers breakage. (3) Bridges occur mainly at MII and usually are not accompanied by paired acentric fragments. Breakage is correlated with asynapsis and appears to result from aberrant behavior of the Y when this chromosome is a univalent. Deficient types have been useful in studying number, location, and mode of action of the male determiners.

WANG, F. H., Department of Botany, University of Illinois, Urbana, Ill. *Embryological development of inbred and reciprocal hybrid Zea mays L.*—Three well-established inbred lines of corn, 38–11, WF9 and K–64 were used for experiments in sib-self pollinations and reciprocal crosses. Before pollination the silks of bagged ears were carefully separated into left and right halves. The pollen used from all plants of the inbred lines was thoroughly mixed. The left half on 8–10 plants was pollinated with the pollen from the foreign line and the right half was sib-selfed. Ears were collected for study of the embryos after suitable intervals. Fifty or more embryos were dissected out and measured individually for width and length with ocular micrometer. The mean volume of each sample of embryos was also obtained for the more advanced stages. The growth curve of the corn embryo follows the S-type. The hybrid embryo is always larger than that of female parent on the same ear. The mean size of the reciprocal hybrid embryos is always larger than the mean of the parents. The difference is observed in the earliest stage beginning eight days after pollination and is maintained until the maturation of the seed.

WENT, F. W., California Institute of Technology, Pasadena 4, Calif. *Thermoperiodicity in plants.*—In plants the daily change from light to dark is attended by a change of processes limiting development. Whereas during the photoperiod photosynthesis presumably limits subsequent growth, during the nyctoperiod other processes control further development. Since the light and dark processes have different temperature characteristics, the optimal temperature during the day is usually different from the optimal temperature during night. In all plants investigated thus far the optimal night temperature is lower than the optimal phototemperature, which parallels the conditions to which the plants are exposed in nature.

Whereas for a number of plants flowering and fruit set are controlled by the *length* of the photoperiod (or rather nyctoperiod), in others dry or wet weight production or fruit set is controlled by the *temperature* during the nyctoperiod. Therefore it is of importance to study thermoperiodicity in greater detail. It is likely that the distribution of plants according to altitude and latitude is at least partly determined by their thermoperiodic response, and the habit of plants is strongly affected by the sequence of day and night temperatures.

WILLIAMS, BERT C., University of Alabama, University, Ala. *Differentiation of tissues in root tips.*—In a survey of root tip structure, including 195 species of vascular plants, the cylindrical layer of cells which surrounds the plerome and which later differentiates into the endodermis has been found to act like a cambium giving rise to the entire cortex in the primary root tip of all vascular plants. Lateral root initials have been found in the pericycle of the meristematic root tips of seven hydrophytic plants (five monocots and two diocots), as well as in the endodermis of the root tip of *Marsilia*. Primary sieve tubes, except in gymnosperms, consistently appear differentiated and functional down into the region where cell walls are thin, and growth and cell division is very rapid. In five species of composites intercellular canals parallel the sieve tubes immediately outside the endodermis and seem to be a part of the morphological unit which includes the primary phloem.

WITKUS, E. RUTH, Biological Laboratory, Fordham University, New York, N. Y. *Naturally occur-*

ring polyploid mitosis in the normal development of Allium cepa.—In the developing seedling of *Allium cepa,* polyploid mitotic divisions were found in the cortex cells of the cotyledon. They occurred throughout the cotyledon in seedlings approximately 15 mm. in length. In seedlings about 20 mm. long these divisions were concentrated at the base of the cotyledon. In seedlings longer than 20 mm. polyploid divisions were very scarce.

In the formation of these polyploid cells a double reduplication takes place in the long resting stage preceding the division. Evidence for this was obtained from the fact that in the earliest prophase stages the chromosomes consist of four chromatids held together by a single undivided spindle attachment region (tetra-chromosomes). At metaphase two successive divisions of the spindle attachment region occur. After the first division, the tetraploid nature of the cell becomes apparent, and the chromosomes are arranged in pairs. The second division of the spindle attachment region results in the separation of chromatids, which then undergo normal anaphase movement. Two tetraploid cells result. The polyploid cells probably undergo only one division, since only metaphases with paired chromosomes were found. If the cells divided more than once this paired arrangement would have been disrupted.

Woods, M. W., and H. B. DuBuy, University of Maryland, College Park, Md., and Berwyn, Md. *Further studies on plastid variegations.*—As previously shown many leaf variegations are caused by abnormal plastids or plastid-forming mitochondria. Twenty-six variegated species or varieties representing nineteen genera have now been cytologically investigated. These variegations can be grouped so as to form a range of types showing progressively more virus-like symptom patterns. Gradations in macroscopic symptoms which are often paralleled by decreases in size and structural complexity of the variegation-inducing agents are illustrated within a single genus (*Hedera*). These abnormal plastid derivatives are constantly associated with pathological changes in the cells containing them, and are often fundamentally like those caused by typical mosaic viruses.

PALEOBOTANICAL SECTION

(Abstracts of this Section are arranged alphabetically according to author)

Andrews, Henry N., The Missouri Botanical Garden, St. Louis, Mo. *The Idaho Tempskyas.*—During the past four years the author has made two collecting trips into southeastern Idaho primarily in search of *Tempskya,* a genus of tree ferns now known to have been quite abundant through certain parts of northwestern United States during Upper Cretaceous times. In September of 1945 a considerable number of specimens was obtained in the vicinity of Wyan which add appreciably to our knowledge of the general habit of these plants. Associated petrified remains were also found which suggest that the Tempskyas grew in association with conifers, dicotyledons, and cycads.

Arnold, C. A., University of Michigan, Ann Arbor, Mich. *The relative merits of cellulose peel and petrotome sections in paleobotanical research.*—The advantage of the cellulose peel method in preparing thin sections of plant petrifactions is the saving of labor and material. A disadvantage is that peel sections are seldom if ever as good as sections made by the other method, and in material where preservation is a limiting factor in interpretation, it is important that the technique employed in sectioning does not further obscure the cell and tissue structure. Peels are useful for preliminary studies but they should whenever possible be supplemented with well prepared ground sections. A case in point arises in connection with the Cretaceous fern *Tempskya* in which the presence of stone cells in the inner cortex is a specific character. The etching process destroys the thick walls of the stone cells, and in certain instances where ground sections indicate one species, peel sections might indicate another.

Elias, Maxim K., Geological Survey, University of Nebraska, Lincoln, Neb. *Fossil symbiotic algae.*—Since the first suggestion by the author and G. E. Condra (1942) that *Archimedes* and related Paleozoic bryozoa are intimate consortia of two organisms, i.e., a bryozoan and a *phytomorph* (probably an alga), extensive research was conducted among living algae and bryozoa comparable to the Paleozoic organisms in question (results reported at the 1944 meeting of AAAS at Cleveland). Similar fossil consortia were discovered (1945) of a *Polypora* and a phytomorph, building together a double-basket, *Bicorbis,* much in the same fashion as in the living consortium of a sponge (of Chalinea group) and an alga, *Marchesettia spongioides.* In fossils previously described as *Thamniscus* and *Acanthocladia* from the late Paleozoic of America and Salt Range of India the heavy encrusting tissue on the back of the bryozoan colony was found to be similar to *Archaeolithothamnium,* a coralline alga. Investigations also disclosed a large number of *Palaeocoryne* and related complex "appendages" in American Mississippian bryozoan *Fenestella.* These "appendages," previously known only from England, were at one time interpreted by zoologists as parasitic tubularian hydroids (Duncan, Jenkins) but arbitrarily considered by paleontologists as "appendages" (Young and Young) or serving propagative functions (Vine) of the bryozoa. However, they resemble the living Red Alga *Asparagopsis armata.* Now, a new Mississippian fossil has been discovered which looks much like a fenestrate bryo-

zoan, but has no zooecia in it, is composed of filamentous *phytomorphic* tissue, and is attached at one point to a bryozoan, which it mimics.

ELIAS, MAXIM K., Geological Survey, University of Nebraska, Lincoln, Neb. *Taxonomy of Tertiary flowers and herbaceous seeds.*—The taxonomy of Tertiary flowers presents almost insurmountable difficulties because of invariably incomplete preservation of the tender stamens and pistils. Whorled and not spirally constructed, flower units are by far the prevailing type among late Tertiary flowers, and are the only ones known among Eocene and Cretaceous flowers. The best known Tertiary herbaceous seeds belong to the Cyperaceae, water-soldier (*Stratiotis*), Boraginaceae, and, particularly, to grasses of the tribes Stipeae and Paniceae. Because seeds of the related living herbs are given much consideration in taxonomy, the same principles of differentiation and taxonomic treatment are fully applicable to living and fossil seeds, when the latter are satisfactorily preserved. The fertile glumes, which make the outer cover (hull) of a grass seed, are also homologous to the calyx of ordinary flowers and do not change in maturity. The American early Miocene hulls of Stipeae show essentially the same structure as in the living Stipeae, plus some details, which are lost in most living species. Preservation of all fine details in the Tertiary hulls of Stipeae and the Borage nutlets invites additional study and detailed illustration of the same in their living descendants, and promises better understanding of their evolution and improvements in their taxonomy.

FENTON, CARROLL LANE, Rutgers University, New Brunswick, N. J. *Records of early algae.*—Fossils of Pre-Cambrian and early Paleozoic algae consist chiefly of carbonized films and laminae, impressions, and dolomitic structures of branched, sheetlike or nodular form. Although once the subject of considerable argument, the algal nature of these fossils may now be accepted. They indicate a considerable variety of species, among which massive forms belonging to the red algae were dominant in many habitats. As yet, however, the fossils do not indicate any orderly sequence of types, nor do they give clues to evolution within the various groups represented.

HANSEN, HENRY P., Oregon State College, Corvallis, Ore. *Postglacial vegetation of the northern Great Basin.*—The northern portion of the Great Basin extends into south central Oregon, which is characterized by closed fault basins with interior drainage. The faulting probably occurred during the Pleistocene and the basins were filled with water during the remainder of the glacial epoch. As the waters became shallower, organic sediments accumulated, which bear a pollen record of the adjacent vegetational changes during the postglacial. Three sedimentary columns were obtained from this region. Two of the sections contain an interbedded stratum of pumice from the eruption of Mount Mazama which formed the caldera holding Crater Lake in southern Oregon. The third section rests directly upon the pumice. The pollen analytical data indicate that the early postglacial forests consisted of *Pinus monticola, P. contorta,* and *P. ponderosa,* with a preponderance of the first. Appreciable areas of grasses, Chenopods, and Composites must have existed nearby, as they are represented early in the columns. As the climate became warmer and drier *P. monticola* was replaced by *P. ponderosa* which in turn was partially replaced by grasses, Chenopods, and Composites during the warm, dry period between 8,000 and 4,000 years ago. A contraction of areas covered by grasses, Chenopods, and Composites occurred during the last few thousand years, which with a concurrent increase in *P. ponderosa,* reflects a cooler and wetter climate in more recent time. The stratigraphic position of Mount Mazama pumice in relation to the trend of forest succession denotes that the eruption of Mount Mazama occurred before the warm, dry interval, but sometime after the maximum of the last Wisconsin glaciation. The date of the eruption is set at about 10,000 years ago.

HOSKINS, J. H., AND A. T. CROSS, University of Cincinnati, Cincinnati, Ohio, and University of Notre Dame, Notre Dame, Ind. *Some basic problems in the classification of paleozoic seeds.*—It is recognized that, because of numerous variations possible in external appearance due to the degree and mode of preservation of any given seed, it may exhibit one of several fossil forms, to each of which a different name is assignable. Petrified seeds of known generic rank, as based on adequate anatomical features, may so resemble each other in external appearance, due to original form or subsequent variations in preservation, that they are indistinguishable. Thus in the instances where adequate anatomical details are lacking they are referable to the same genus. Therefore the use of generic terms for seeds showing adequate internal organization which are different from those names assigned to seeds possessing only external or superficial characteristics is a necessary basis for taxonomic work on Paleozoic seeds.

The internal organization of a new species of *Pachytesta* is given to illustrate the several possible variations in external form and appearance due to varying degrees of decortication during fossilization. At the present time there appear to be nine valid genera of the Trigonocarpales based on anatomical characteristics. Five genera are conserved and redefined to include seeds of this order which are identifiable only by means of external or superficial features.

JOHNSON, J. HARLAN, Colorado School of Mines, Golden, Colo. *Late Paleozoic algae of America.*—The paper presents a brief general resumé of our knowledge of late Paleozoic algae. The data are presented systematically according to the character of the algae. Charts show the known genera and their distribution, both by age and geographically. At present Devonian algae are practically unknown

in North America, and only a few finds have been recorded from the Mississippian. Algae appear quite commonly in the Pennsylvanian of Kansas, Colorado, Oklahoma, New Mexico, and Texas. Permian forms are locally abundant and some are widely distributed. Some of the problems awaiting study are mentioned.

JUST, THEODOR, University of Notre Dame, Notre Dame, Ind. *The relative value of taxonomic characters.*—Experience has demonstrated the great practical value of many taxonomic characters in the identification of some groups and their failure in others. Actually the value of taxonomic characters may not only vary from group to group but also with different systematic categories. In addition, the nature and purpose of particular classifications affect greatly the selection and usefulness of taxonomic characters. Consequently in difficult groups either new taxonomic characters must be found or known characters must be restudied in as many samples as possible or both must be combined for a satisfactory interpretation of taxonomic complexes. Modern methods of analysis provide taxonomists with many new data, i.e., definite or constant characters needed for the delimitation of taxonomic groups and variable characters constituting so-called character-gradients valuable for possible subsidiary classifications.

O'HANLON, SR. M. ELLEN, Rosary College, River Forest, Ill. *Leaf form in ontogeny and phylogeny.*—The results of experiments and observations on the superficial characters of the foliage leaves of certain families of the angiosperms raise a number of questions. A comparison of the ontogenies of several species with the conclusions, theories and speculations which are recorded by taxonomists and paleobotanists relative to the phylogenies of these various alliances is also of considerable interest and significance.

In certain families and closer alliances of the angiosperms there is a rather wide difference in foliage leaf form and development. Moreover the ontogeny and the putative phylogeny of such leaves are difficult to explain and to reconcile in some cases even within a single family. For example, the direction in which evolution of the foliage leaf seems to have advanced in one species is sometimes exactly opposed to that of another species within a family or even within a genus. The complexity of the whole problem is rather augmented when a comparison of the ontogeny and of the phylogeny of both the foliage and the floral leaves is considered.

In some well-known species both the foliage and the floral leaves seem to have persisted apparently unchanged throughout geologic time; in others both types of foliar organs have evolved in apparently diverse directions; there are some species too in which one or the other type of foliar structures has evolved while the corresponding structures have remained static.

RADFORTH, NORMAN W., University of Toronto, Toronto, Canada. *The taxonomic treatment of the fern—Pteridosperm complex.*—Taxonomic difficulties encountered as a result of analyses of fossil fern-like plants are considered. In the light of these and of modern trends in taxonomy the significance of the designation Pteridospermae is reviewed.

WILSON, L. R., Coe College, Cedar Rapids, Iowa. *Pollen evidence for tundra in the vicinity of Lake Superior and James Bay.*—In the United States no definite proof is at this time known from peat pollen studies for the existence of tundra at the time when the Pleistocene ice was melting. All basal deposits in peat bogs contain pollen of forest trees. This is considered to indicate that trees in the United States migrated northward almost as fast as the glacial ice wasted. Anticyclonic winds from the glaciers probably prevented pollen from being transported from more southern localities.

In the vicinity of Lake Nipigon, and Cochrane, Ontario, peat bogs have been found that contain no tree pollen in their lowest levels. The peat is of tundral type, consisting of grasses, sedges, and mosses. It appears that the present tree line was extended northward from a position somewhere to the south of these bogs, after glacial Lake Objibway time.

PHYSIOLOGICAL SECTION

(Abstracts of this Section are arranged alphabetically according to author)

BARNES, T. C., Hahnemann Medical College and Hospital of Philadelphia, Philadelphia, Pa. *The growth-stimulating action of chlorophyll on human skin in experimental abrasions.*—Skin on the fingers was excoriated with autoclaved sandpaper and the positive wound potential of 15 to 40 millivolts measured by a recording potentiometer (for methods see Barnes, Am. J. Surgery 69: 82, 1945). As the skin grew back over the abrasion the wound potential fell giving the healing index or per cent loss of wound potential per hour. Skin treated with 0.2 to 1.0 per cent chlorophyll had a growth rate of 2.43 $\pm$ 0.37 per cent per hour compared with a rate of 1.13 $\pm$ 0.27 in control wounds on the same hand treated with the vehicle used (saline, petrolatum or synthetic phenolic resin). It is concluded that chlorophyll stimulates growth of animal tissue but not as strongly as extracts of yeast and fish liver (see reference given above).

BARTON, LELA V., AND HELEN R. GARMAN, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y. *The effect of age and storage condition of seeds on the yield of certain plants.*—Plants were grown in the field from fresh and old seeds of the same genetic strains of aster, verbena, lettuce, pepper, and tomato. Data were taken on

the flowers produced by aster and verbena, size of heads produced by lettuce, and weights of fruits from the pepper and tomato plants. No differences were found in the behavior of plants from nine-year-old as compared with fresh seeds of aster and verbena. Thirteen-year-old seeds of pepper produced plants with as high yield of fruit as fresh seeds. Plants from tomato seeds stored for thirteen years under favorable conditions were as good in every respect as those from fresh seeds. Thirteen years' storage under unfavorable conditions delayed the germination of tomato seeds with subsequent slow development of the plants in the field resulting in reduced yield of fruit. There was evidence that lettuce heads from thirteen-year-old seeds were superior to those produced from fresh seeds.

BECK, EDWIN G., Hannibal-LaGrange College, Hannibal, Mo. *A study of the Solidago gall caused by Eurosta solidaginis.*—The gall is formed as the result of cell division in the pith region and the meristematic activity of "meristematic strands" which originate from the cambium and xylem regions of the stem. The abnormal growth is induced by a stimulus initiated by the presence of the maggot of *Eurosta solidaginis* in the stem. The excrement of the maggot is found to contain amylase, invertase, and protease, to be alkaline, and to increase the pH of artificial media and of normal *Solidago* stems in which the maggot lives. Oxidizing enzymes, protease, amylase, proteins, and glucose are found to be most abundant in the region surrounding the larval cavity, in the meristematic strands, and cambium and to occur in smaller amounts or not at all in other regions of the gall. The pH values of the layer of cells lining the maggot's chamber and of the tissues of the meristematic strands are found to be higher than those of normal stem tissues. Extracts of gall tissues, maggot cultures and maggots yielded no materials which would induce gall formation in the *Solidago* stem. Some gall formation was induced by injections of trypsin, mixtures of amino acids, and protein digests. It is concluded that the proteolytic enzymes and the elevated pH values are important factors in this case of gall formation.

BERGER, J., PATRICIA SMITH, AND G. S. AVERY, JR., Brooklyn Botanic Garden, Brooklyn, N. Y. *The influence of auxin on respiration.*—The respiration of living tissue from the *Avena* coleoptile (in 1 per cent sucrose solution) is stimulated by about 35 per cent in the presence of 10 mg./l. of indoleacetic acid. This confirms the work of Commoner and Thimann (1941). Tissues starved of excess carbohydrate substrate, and depleted in auxin content, give a stimulated respiratory rate in sucrose solution when indoleacetic acid alone is added. Simultaneous addition of the four-carbon acid, malic, does not catalytically increase the oxygen uptake.

BLINKS, L. R., AND C. M. LEWIS, Stanford University, Calif. *Rapid recording of the time course of photosynthesis.*—Electrical means of following the time course of the gas exchange in photosynthesis has been employed, using a glass or antimony electrode for the pH changes caused by CO_2 utilization (or production); and a polarographic oxygen reduction method for the increase (or decrease) of dissolved O_2. The tissue is in direct contact with the electrode, reducing diffusion distance to a minimum. Photographic recording of galvanometer deflections, or (with amplification) of cathod ray oscillograph traces, permit continuous recording of the effects of single light flashes as short as 0.001 second. This has been done with leaves, algal thalli, films of unicellular algae, and extracted chloroplasts. The method is particularly useful in indicating the changes during the so-called induction period following long dark periods, and has shown several unsuspected anomalies, including an initial O_2 "gush," followed by a recession to low oxygen evolution, or even of oxygen consumption under some conditions. Anomalies in the CO_2 exchange are also present. The effects of temperature, O_2 and CO_2 concentrations, light intensity, poisons, and repeated flashes separated by varying dark periods have been studied, and are illustrated by characteristic records.

BROWN, ALLAN H., University of Chicago, Chicago, Ill. *Carbohydrate formation in Scenedesmus obliquus.*—Analyses were made of the carbohydrate fractions of cells of *Scenedesmus obliquus* (strain D_1) grown in inorganic media under constant illumination. By minor modifications of conventional procedures, washed cells were extracted in turn with hot 80 per cent ethanol, hot water, and the residue hydrolyzed with acid to separate alcohol, soluble sugars, water soluble polysaccharides, and water insoluble polysaccharides. The principal carbohydrate was found to be a starch-like constituent. This is in agreement with previous reports of starch as a storage product in other *Chlorophyceae*. Little water soluble polysaccharide was present (less than 1 per cent of dry wt.). Generally only small amounts of free reducing sugar were found (½ per cent or less). Sucrose was the major constituent of the alcohol fraction and was specifically identified by invertase hydrolysis with subsequent quantitative determinations of aldose and ketose. Pentoses were absent. The yield in the several fractions depended upon culture conditions. Old and crowded cultures contained more sucrose than young cultures on a dry weight basis. However, the mobility of the photosynthate between fractions was less than might have been anticipated. For instance, respiration for 24 hours in the dark, with aeration, only slightly altered the fractionation results by producing a slight depletion of sucrose and a very small increase in free reducing sugar.

CHAMBERS, ROBERT, New York University, New York, N. Y. *Vital coloration properties of methyl red.*—Methyl red is of considerable significance to the cytologist because of the peculiar properties which condition its penetration into living cells. In the normal media of animal cells, e.g., seawater for

starfish eggs, and pond water for plant cells, such as the root hair cells of *Limnobium,* methyl red does not penetrate the cells. However, on micro injection into the cytoplasm of the starfish egg a yellow color spreads and accumulates in the nucleus (germinal vesicle). When injected into the vacuole of the root hair cell, the color which is reddish yellow, passes into the cytoplasm where it takes on a yellow color and accumulates in the nucleus. Eventually, the dye passes more or less completely out of the cytoplasm into the nucleus and into the medium. Vital staining can also be induced from the external medium by acidifying the latter as low as possible within viable limits. Methyl red was originally included in the Clark and Lubs series of pH color indicators. It is amphoteric, forming, on the acid side of its isoelectric point (ca pH 4.4) the chloride of a red colored base and, on the basic side, a sodium or potassium salt of a yellow colored acid. Thus, according to the pH of the medium, methyl red can serve either as a basic or as an acid dye.

FARR, WANDA K., Celanese Corporation of America, Cumberland, Md. *Calcium pectate in the cotton fiber.*—The presence of calcium pectate in the primary wall of the cotton fiber is demonstrated in wall fragments separated mechanically from the secondary lamellae. The role of this substance in determining certain physical properties of the fiber is shown by swelling reactions in water and in cuprammonium hydroxide.

FRENCH, C. S., G. S. RABIDEAU, AND A. S. HOLT, University of Minnesota, Minneapolis, Minn. *The design of a large aperture grating monochromator for use in photosynthesis investigations.*—An instrument has been constructed that will give monochromatic light of sufficient intensity for the measurement of photosynthesis in red light with a reasonably narrow spectral band width. The apparatus uses a $4'' \times 6''$ replica grating and large condenser lenses of $17\frac{3}{4}''$ focal length. It covers the spectral range of 350 to 1000 mμ. The dispersion is 3.55 mμ./mm. in the second order and 7.1 mμ./mm. in the first order. The entrance and exit slits are fixed on axes at 90° to each other and the wavelength of the emergent beam is controlled by the rotation of a mirror and one lens. The per cent transmission of the instrument reaches a maximum of 34 per cent in the red of the first order and 21 per cent in the blue region of the second order. The entrance slit is 34 mm. long and the exit slit 50 mm. long. A small cylindrical lens near the entrance slit reduces the vertical height of the beam entering the grating thus increasing the output by about 20 per cent. At 650 mμ. with 2 mm. slits it will isolate from a tungsten source 3.7×10^{-2} cals./min. with about 95 per cent of the energy within a band 28 mμ. wide. The apparatus has been used for several years for the study of the efficiency of oxygen evolution by chloroplasts and for the measurement of leaf absorption and reflection spectra with an Ulbricht sphere. The parts are not expensive.

GARMAN, HELEN R., AND LELA V. BARTON, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y. *The response of lettuce seeds to thiourea treatments as affected by variety and age.*—The stimulatory effect of soaking lettuce seeds in thiourea was found to depend not only upon the variety but upon the length of the period of dry storage of the seeds after harvest. Increasingly beneficial effects on germination at 30°C. were obtained with extension of the dry storage period from immediately after harvest to sixteen weeks. This was true for the four varieties studied, Grand Rapids, Iceberg, White Boston, and Black Seeded Simpson, though the germination percentages obtained varied.

GOODWIN, RICHARD H., AND OLGA OWENS, Connecticut College, New London, Conn. *The effect of monochromatic light on chlorophyll formation and internode inhibition in Avena.*—Studies are being made on etiolated seedlings of *Avena sativa* to determine the relative effectiveness of various wave lengths of monochromatic light (1) in inducing chlorophyll formation and (2) in inhibiting the elongation of the first internode. The results thus far obtained indicate that the blue and red regions of the spectrum are the most effective both in the production of chlorophyll and in internode inhibition. Chlorophyll *a* appears to be the first of the chlorophyll components formed after irradiation. Spectrophotometric analysis of the chlorophyll produced during the first two hours of exposure gives no indication of the presence of chlorophyll *b*.

GORDON, SOLON A., Institute of Tropical Agriculture, Mayagüez, Puerto Rico. *The effect of auxin on the soluble-nitrogen levels in red hibiscus.*—Following the experiments reported on by van Overbeek, it was found that the stem and leaves, as well as the base, of auxin-treated hibiscus cuttings possessed higher soluble-N levels than controls. Detached leaves also showed marked increases of soluble-N after auxin treatment. That auxin conditions the activity of proteolytic enzymes seems, therefore, a reasonable possibility. Preliminary experiments on the effect of auxin on hibiscus proteases are reported.

HILDEBRANDT, ALBERT C., AND A. J. RIKER, Department of Plant Pathology, University of Wisconsin, Madison, Wis. *Some common sugars, polysaccharides, and organic acids as carbon sources for plant tissue cultures.*—Tissue cultures of marigold, Paris daisy, periwinkle, sunflower, and tobacco were incubated on basal media lacking or containing 1 per cent, respectively, of one of the following carbon sources: arabinose, xylose, rhamnose, dextrose, levulose, mannose, galactose, sucrose, maltose, lactose, cellobiose, raffinose, starch, inulin, dextrin, or pectin. Marigold tissue grew with dextrose, levulose, mannose, sucrose, maltose, cellobiose, starch and dextrin; Paris daisy used dextrose, levulose, sucrose, maltose, cellobiose, raffinose, and dextrin; tobacco tissue employed dextrose, levulose, mannose, sucrose, maltose, lactose, cello-

biose, raffinose, starch, dextrin, and pectin; sunflower tissue utilized dextrose, levulose, sucrose, maltose, lactose, cellobiose, and raffinose; and periwinkle tissue grew on dextrose, levulose, mannose, galactose, sucrose, maltose, lactose, cellobiose, raffinose, starch, dextrin, and pectin. The tissue cultures failed or grew poorly on media containing the other sources of carbon. None grew in the light or in darkness without a source of carbon. The 5 species failed or grew poorly without sugar but with 0.5 per cent, respectively, of acetic, citric, malonic, malic, succinic, formic, tartaric, pyruvic, propionic, oxalic, stearic, glycolic, or lactic acids, or calcium gluconate. However, with these acids and 2 per cent sucrose the 5 species grew in the presence of malic, succinic, and stearic acids, and calcium gluconate; marigold used also malonic, tartaric, glycolic, and lactic acids; Paris daisy, malonic acid; tobacco and sunflower, malonic and tartaric acids. The 5 species failed or grew poorly on the other organic acids.

Hitchcock, A. E., and P. W. Zimmerman, Boyce Thompson Institute, Yonkers, N. Y. *Hormone effects and ultimate death of plants caused by various growth substances.*—Plants were sprayed with different substances at about 1 g./l. using principally 1 per cent polyethylene glycol 400 mono oleate as a carrier. According to hormone responses and ultimate death, NA-salts or esters of 2,4-dichlorophenoxyacetic acid (2,4-Cl_2POA), 2,4-Cl_2POB, 2,4,5-Cl_3POA, 2,4,5-Cl_3POB, 2-CH_3 4ClPOA, and 2-$CH_3$4ClPOP were most active and of nearly the same effectiveness on dandelion, plantain, chickweed, hawkweed, mustard, lawn pennywort, Creeping Jennie, self-heal, tomato, and tobacco. *Nepeta hederaceae* was killed consistently only by 2,4,5-Cl_3POA. Relative toxicities: 2,4,5-Cl_3POA > 2,4-Cl_2POA for killing white clover and *Oxalis;* 2,4,5-Cl_3POA > ethyl ester of same and 2,4-Cl_2POA < ester for killing tomato and clover; 2,4,5-Cl_3POB > 2,4-Cl_2POB > 2-ClPOB and all three greater than corresponding acetic and propionic homologs for inhibiting germination and subsequent growth of white clover planted 2 to 20 days after spray treatment. Sensitivity of plants was greater in soil than in sand. Two component mixtures particularly of 2,4-Cl_2POA, 2,4,5-Cl_3POA, or 2-$CH_3$4ClPOA were more effective than individual components, especially 2,4,5-Cl_3POA + 2-$CH_3$4ClPOA. Some growth substances, particularly substituted benzoic acids, were more effective when added to soil than when sprayed on the plants. All plants (including blue grass and bents) which responded to concentrations of about 1 g./l. exhibited hormone responses regardless of whether ultimately killed. Effectiveness of sprays on given plants depended upon environmental factors, carrier, dose, and method of spraying.

Kelly, Sally M., Brooklyn Botanic Garden, Brooklyn, N. Y. *The relation between respiration and water uptake in the Avena coleoptile.*—Through the use of inhibitors a relationship between respiration and water uptake in oat coleoptile segments was established. Respiration, measured as the rate of oxygen uptake, and water uptake, measured as the increase of fresh weight over initial weight, were inhibited to approximately the same extent when soaked in buffer solutions containing different concentrations of several inhibitors. Oxygen uptake was inhibited slightly earlier than water uptake, indicating that the latter was not entirely dependent upon aerobic respiration.

Cyanide, azide, and iodoacetate were the most effective inhibitors, since they inhibited both processes in lower concentrations and in a shorter time than did phlorizin, urethane, pyrophosphate, and malonate.

Lilly, Virgil Greene, and H. L. Barnett, West Virginia University, Morgantown, W. Va. *The influence of pH and certain growth factors on mycelial growth and perithecial formation by Sordaria fimicola.*—On a medium composed of glucose, casein hydrolysate, fumaric acid, salts and biotin (6.4 micrograms per liter) the rate of mycelial growth and the total dry weight of mycelium produced are controlled by the original pH of the medium. Acid is produced during the early period of growth. If the original pH of the medium be below 4 the fungus soon ceases to grow unless thiamin is added to the medium as an accessory growth factor. The pyrimidine moiety replaces thiamin *per se* as an accessory factor.

With no thiamin added to liquid cultures (25 ml. medium per 250 ml. Erlenmeyer flask) at an original pH of 6.0 perithecia formed in 13 days, at an original pH of 4.0 perithecia formed in 19 days; whereas if the original pH were 3.8 or less perithecia did not form within 60 days. Addition of thiamin to the medium at original pH of 3.8 and 3.6 allowed perithecia to form in 17 and 42 days respectively. The number of perithecia formed, as well as the time of their appearance, is also a function of the biotin content of the medium. Suboptimal biotin concentration has a greater influence upon the number of perithecia formed than upon the final weight of the mycelium produced.

Myers, Jack, University of Texas, Austin, Tex. *The application of a continuous-culture technique to problems in the physiology of Chlorella.*—A continuous-culture apparatus (previously described) dilutes an algal culture just as fast as it grows so that it is held always at one point on its growth curve. Environmental conditions are maintained constant in any one culture which produces uniform experimental material day after day. The effect of culture conditions can be examined by studying a single variable at a time. All effects of growth on environmental conditions are eliminated and growth rate may be determined accurately under constant conditions, an accomplishment not possible in a freely growing culture.

Certain cellular characteristics, photosynthetic characteristics, and growth rate have been studied

in relation to a number of culture conditions. Data are presented on the effects of varying the population density, the pH, the total concentration of the Knop's solution, the iron concentration, and the concentration of micronutrients. In response to any of these conditions *Chlorella pyrenoidosa* is not particularly sensitive. It is concluded that other conditions (e.g., light intensity, carbon dioxide concentration) may be studied without danger of disturbing effects by any small variations in population density or composition of the nutrient medium.

MYERS, JACK, University of Texas, Austin, Tex. *Influence of light intensity of culture upon cellular and photosynthetic characteristics of Chlorella.*—*Chlorella pyrenoidosa* was grown in the continuous-culture apparatus with light intensity provided by tungsten lamps the only variable between different cultures. Analyses on harvested cells were made for cell number, dry weight, nitrogen, and chlorophyll per unit cell volume. Data are plotted against light intensity of culture. An unexpected result is that both chlorophyll concentration and cell size vary over a 5-fold range and in such a way that there are always about the same number of chlorophyll molecules per cell.

Light intensity curves of photosynthesis (cmm. O_2/hour/cmm. cells) are presented for cells cultured under different light intensities. Cells grown at low intensities give a Blackman type curve with a short transition region. Cells grown at high intensities show a long transition region and become light-saturated only at high intensities. Plotting maximum rate of photosynthesis attainable (Blackman rate) against light intensity of culture reveals that maximum capacity for photosynthesis is developed by cells grown in a narrow range of light intensity at about 35 f.c. A fall-off in capacity for photosynthesis above 50 f.c. light intensity of culture seems to be correlated with attainment of a maximum growth rate. The growth rate shows a sharp light-saturation not determined by photosynthesis. At light intensities of culture below 35 f.c. capacity for photosynthesis falls off rapidly, a phenomenon not yet explainable.

RABIDEAU, G. S., C. S. FRENCH, AND A. S. HOLT, University of Minnesota, Minneapolis, Minn. *The absorption and reflection spectra of leaves and chloroplast suspensions.*—The absorption spectra of leaves and chloroplasts isolated from leaves were measured with the monochromator and Ulbricht sphere photometer described by French, Rabideau, and Holt. Absorption peaks were found in the red region at 670 mμ and in the blue region at 500 mμ, although the latter was not sharply defined. Absorption minima were found at 550 mμ and in the near infrared region, 700-850 mμ. The thinner leaves showed more clearly defined bands than did thick leaves. Reflection maxima occurred at 550 mμ and again at 700–850 mμ. At the red peak, the absorption varied between 80 and 95 per cent for various species of leaves. In the near infrared the absorption varied from 0–8 per cent. The reflection maxima were 50–75 per cent for the near infrared and 35 per cent for the visible region. Chloroplasts isolated from leaves and suspended in sucrose solution showed the same absorption and reflection maxima as did intact leaves, but the bands were more clearly defined. The clear green supernatant fraction obtained by centrifugation of chloroplasts ruptured by supersonic vibration showed the same location of absorption maxima and minima, and the bands were sharper than with the intact leaves or with the chloroplast suspensions.

RIKER, A. J., AND ALICE E. GUTSCHE, University of Wisconsin, Madison, Wis. *Growth in vitro of sunflower tissue inhibited by ammonia and various amino acids.*—Sunflower tissue from a "secondary" crown gall was studied on synthetic media with varying nitrogen sources. Four tissue fragments weighing about 20 mg. and placed on 40 ml. of media in a 6-ounce bottle comprised 1 of over 1300 cultures. After 6 weeks at about 26°C. their wet weights were recorded.

On geometrically increased concentrations of sodium nitrate, the tissues grew well between 0.002 and 0.064 molar concentration. Neither ammonium chloride nor ammonium tartrate alone supported growth and along with 0.008 M. nitrate both reduced growth to poor at about .004 and 0.001 M., respectively, and to none at 0.064 M. Sodium nitrite alone permitted little or no growth at any concentration; at more than 0.001 M. it prevented growth on nitrate. On urea between about 0.016 and 0.064 M., the tissues grew well with or without nitrate.

Geometrically increased concentrations of amino acids with nitrate constant at 0.008 M. gave the following results. Glycine, dl-β-phenylalanine, and dl-valine progressively inhibited growth to little or none on stronger than 0.001 M. dl-Alanine, l(+) aspartic acid and l(+)glutamic acid progressively inhibited growth with low points at approximately .001, .004, and .008 M., respectively; however, at still stronger concentrations each of these 3 compounds permitted fair growth at about .064 M. The inhibition observed suggested that certain simple nitrogenous compounds deserve further consideration as regulators or inhibitors especially of indeterminate normal or pathological growth.

ROBERTS, EDITH A., Vassar College, Poughkeepsie, N. Y. *Electron microscope studies of plant cells and their contents showing structural and functional units of less than 100 ängströms.*—Electron microscope studies of the chloroplast of *Anacharis canadensis; Gladioli* sp.; *Pyrus Malus; Nicotiana Tabacum;* and *Oedogonium* sp. show that they are made of units of less than 100 ängströms. Studies of chlorophyll "crystals" show that they are not crystals but are units of less than 100 ängströms.

Virus-infected *Nicotiana Tabacum* shows that the entities comparable to what have been called "viruses" appear to be disintegrating plastids. They are less than 100 ängströms and are not crystalline.

Studies of "alpha carotene" and "beta carotene" in *Mycobacterium phlei, Allomyces javanicus* var.

macrogyna, and *Daucus carota* show that the carotin is not in crystals, but in units of less than 100 ängströms.

"Crystalline" vitamin A is of units less than 100 ängströms and is not crystalline.

Colloidal carbon is made of units less than 100 ängströms. These are comparable to those units found in plant walls from which colloidal carbon has been derived and appear to be dehydrated protoplasmic entities.

The above show a relationship of chloroplasts to viruses, chromoplasts to carotin bodies, carotin bodies to vitamin A, and cellulose to colloidal carbon.

These protoplasmic structural and functional entities of plant cells and their contents are smaller than any protoplasmic entities reported to date. In the anabolic as in the catabolic processes units less than 100 ängströms are functional within the plant and their size with their surface values have unlimited applications in the use of plant materials.

SHALUCHA, BARBARA, Brooklyn Botanic Garden, Brooklyn, N. Y. *Extraction of auxin from green plant tissue.*—Maximum auxin yields from young shoots of peach (*Prunus persica,* var. South Haven) have been obtained on autoclaving for two hours in 1N alkali followed by an ethyl ether extraction. In this green tissue (dried at low temperature and pressure), about 5–10 per cent of the total extractable auxin is free and water soluble, the other 90–95 per cent remains as precursor, but is converted to auxin on alkaline hydrolysis. Reproducible *Avena* assays are made possible by re-extracting the treated aqueous suspension with ethyl ether.

The auxin of peach shoots is stable to strong alkali and heat. The precursor is insoluble in ethyl ether and chloroform, but partially soluble in water and methanol.

The maximum auxin content in peach shoots was found during the period of most rapid increase in shoot length.

Total nitrogen determinations were made along with the auxin studies.

SMITH, HAROLD H., Cornell University, Ithaca, N. Y. *Some quantitative aspects of aqueous spray applications of 2,4-dichlorophenoxyacetic acid for herbicidal purposes.**—Experiments were carried out to investigate relationships between the plant response induced by sprays containing growth-inhibiting compounds and certain characteristics of the sprays as volume rate, concentration and amount of compound, droplet size and degree of interception. Sprays were more effective on young kidney bean plants when applied at rates of 10–20 ml. per sq. yd. than in larger or smaller volumes. With further reductions in spray rates below 10 ml. per sq. yd., progressively more agent was required to accomplish the same magnitude of inhibition. Sprays of relatively large droplet size (250–561 μ av. dia.) were more effective than those composed of finely atomized particles (30 μ av. dia.). Studies on interception showed that more solution was intercepted and retained on a plant when sprays of larger droplet size were used. There was also an indication that spray rates of 10–20 ml. per sq. yd. were optimum for highest percentage of interception and retention. The fact that growth inhibitors can be effective when applied in such low volumes opens up new fields for large scale practical application of herbicides.

* Studies conducted at Camp Detrick, Frederick, Md.

SOUTHWICK, MILDRED D., Vassar College, Poughkeepsie, N. Y. *Electron microscope studies of the structure of the starch grain, showing its relationship to plastid structure.*—Electron microscope studies have been made of the structural organization of starch grains from seeds, roots, stems and leaves from several genera of plants, belonging to the Bryophyta, Pteridophyta, and Spermatophyta. These studies indicate the same essential morphology of all starch grains whether from leucoplasts or chloroplasts. Electron micrographs have been made of these structures both in their natural position in the grains and isolated from the grains. All of these show the structural organization of chloroplasts—the larger units, plastidules and the smaller units, plastid granules (Roberts). The plastid granules are shown to consist of still further structural units which approach the smallest functional protoplasmic units known.

TURRELL, F. M., University of California, Citrus Experiment Station, Riverside, Calif. *Averaging pH values.*—Taking an arithmetical average of pH values is an incorrect mathematical procedure but where numerous values are to be summarized it has sometimes been used because of the labor involved in conversion to $[H^+]$. Where the range of pH values is small the arithmetical average may not be in greater error than the probable error; however, while arithmetical means of two sets of pH values with wider variations may be equal, averaging $[H^+]$ value and converting to pH may gives statistically significant values. Mathematically correct procedures are recommended for finding the average pH in order to avoid obscuring significant differences, especially where statistical analyses are to be applied. Use of tables of corresponding pH and $[H^+]$ values designed for use with glass electrode instruments greatly reduces labor in making proper calculations.

VAN FLEET, D. S., University of Missouri, Columbia, Mo. *The sequence of glucosidase and oxidase associated with the development of dermal and perivascular tissues.*—The presence of aglycones in dermal, endodermal and bundle sheath tissues is associated with the localization of glucosidase action. Glucosides are converted to aglycones in all parenchymal cells when β-glucosidase is placed on sections, but in control sections the aglycones are confined to dermal and perivascular tissues. Glucosides introduced into leaves and stems are hydrolyzed, and a deposit of the aglycone may be detected primarily in boundary tissues. The glucoside

amygdalin releases HCN in the endodermis and bundle sheath when sections are made, but if glucosidase is added to the sections there is a release of HCN in all parenchymal cells, indicating a general distribution for the glucoside and a localized distribution for glucosidase in perivascular tissue. The momentary release of HCN inhibits the fat-peroxide oxidation of indicators, and a survey of cyanogenic plants indicates that the fat oxidase system in the endodermis is inhibited. A study of the plants which contain the glucosides, thujin, baptisin, aesculin, chrysanthemin, and phloretin, indicates that glucosidase makes its appearance much earlier than the fat oxidase system in dermal and perivascular tissues. In plants which lack a localized glucosidase, the endodermis does not have a fat-peroxide oxidase system, and the development of the tissue is modified. Glucosidase appears earlier than unsaturated fat and fat-peroxides. The quantity of unsaturated fat, as indicated by iodimetric methods, increases following glucosidase action and the deposition of aglycones.

VAN OVERBEEK, J., Institute of Tropical Agriculture, Mayagüez, Puerto Rico. *The function of the leaf in the process of root formation in cuttings.*—The combined action of auxin and factors coming from the leaves are essential for root formation in cuttings. The leaves exert their influence in the dark as well as in the light. In red hibiscus the root-forming factors of the leaves can be entirely replaced by sucrose and nitrogenous substances such as ammonium sulfate or arginine. Chemical analyses showed that substances of this type are actually contributed to the cuttings by the leaves. It is, therefore, concluded that the main function of leaves in the process of root initiation is to supply the cuttings with sugars and nitrogenous substances, factors which may be termed nutritional. No *a priori* reason was found to postulate the production of a special root-forming substance such as "rhizocaline" in the leaves of red hibiscus.

ZIMMERMAN, P. W., AND A. E. HITCHCOCK, Boyce Thompson Institute, Yonkers, N. Y. *Fluorine compounds given off by plants.*—Results of recent experiments indicate that plants absorb fluorine compounds from the soil and give off volatile fluorine compounds. Leaves absorb hydrofluoric acid from the air when the concentration of the gas is .10 p.p.m. or more of air, thus increasing the normal content of fluorine in tissues. Fertilizers containing fluorine or sodium fluoride applied to soil in which plants were growing caused an increase in the normal fluorine content of tissue. Upon growing for two weeks or more after absorption of fluorine from the soil the tissues lost fluorine as indicated by tissue analyses. It was assumed that the loss was due to escaping volatile fluorine compounds, of which there are many, from plant tissues. This assumption is strengthened by the fact that fluorine compounds accumulate in the air of cases in which plants are enclosed. This accumulation of fluorine varies in amount with the amount of fluorine compounds in the soil of potted plants. The air in the Boyce Thompson Institute laboratories and fields contained variable amounts of fluorine. It is assumed that this comes in part from plants growing in the vicinity. The amount of fluorine in air of forests in Pennsylvania far removed from industries decreased as leaves fell in autumn. During January the air outside Boyce Thompson Institute greenhouses contained less fluorine than that in greenhouses.

SYSTEMATIC SECTION

(Abstracts of this Section are arranged alphabetically according to author)

BABCOCK, E. B., University of California, Berkeley. *The probable center of origin of the genus Pinus.*—The present geographic distribution of the genus indicates one center of origin for both Eurasia and North America. The most ancient pine fossils (Jurassic period) are from northern Eurasia. In the Cretaceous period the genus certainly existed in western Europe and eastern North America; and fossil forms that are apparently intermediate between *Pinus* and *Cedrus* are known from Cretaceous deposits in western Europe. Cretaceous fossils of *Cedrus* are also known from western Europe but, during the Tertiary, this genus was distributed in Europe, Asia, and western North America. The genus *Libocedrus* may have originated in the north, according to Florin, "and then spread not only east and west but also south to New Zealand and southern South America." The present writer suggests that the same may be true of the now widely distributed family Cupressaceae. In *Pinus* both fossil and present-day distributional evidence certainly indicates that the great differentiation of the genus in southwestern United States and Mexico occurred in more recent geological times, i.e., the Tertiary-Quaternary period. Therefore, the most logical inference from available evidence is that the pines originated in northeastern Asia or adjacent Arctica.

BROWN, CLAIR A., Louisiana State University, Baton Rouge, La. *Distribution of alligator weed and its relatives in Louisiana.*—Five species of *Alternanthera*, a genus of the pigweed family, occur in Louisiana. The most abundant of these is the alligator weed, *Altermanthera philoxeroides* (Mart.) *Griesb.*, which is a serious pest in the croplands and waterways. An intensive study of the biology and control of this plant is in progress. This pest occurs in every parish in southern Louisiana and extends up the Mississippi Valley to a limited extent. At present, the northernmost station is in Madison Parish. The 40-knot weed, *A. polygonoides* (L.) Lam., is a procumbent species which is found

as a weed in lawns and waste places. It forms extensive mats around the shore of an intermittent lake in Iberville Parish. This species produces seeds and its common name is suggestive of its rate of spread. Closely allied to this species is *A. repens* (L.) Kuntze, another procumbent species which can be distinguished by its rigid, spine-tipped sepals. *Alternanthera sessilis* (L.) R. Br. is an annual species and is found in Vermilion and Lafayette Parishes. The only collection of *A. leiantha* (Seub.) Standley is from the margin of saline pools in Rayburn Lick, Bienville Parish.

CAMP, W. H., New York Botanical Garden, New York, N. Y. *Biochemical clines, polymorphic populations, and the problems of specific delimitation on the Cinchona population of Ecuador.*—In the Andes of Ecuador at rather definite altitudes and in various geographical areas, morphologically quite well defined and considerably different populations of *Cinchona* may be found; these are sufficiently different to merit recognition as species under the usual criteria. However, after a year and a half of rather concentrated study in the field the conclusion was reached that no effective sterility barriers exist between these populations for, where they now chance to meet, usually complete series of intergrades occur. Some of these apparently hybrid populations have given rise to extensive secondary, independently reproductive groups which at times have been described as species, thereby complicating the taxonomic picture of the genus. A study of the kinds of alkaloids and their relative percentages in a large series of individual trees indicates the presence of large-scale intraspecific biochemical clines as well as somewhat more sharply defined interspecific clines in the areas of polymorphy where two species make distributional contact. Lacking actual breeding data for the present, these morphological variabilities and biochemical characteristics may be used in clarifying the nature and origin of the various major and minor populations and thus begin the stabilization of the systematics of the genus.

DUNCAN, WILBUR H., University of Georgia, Athens, Ga. *Studies of plant distribution in Georgia.*—The probable absence from Georgia of previously reported *Quercus imbricaria* Michx. and *Picea rubens* Sarg. is discussed. The occurrence of a mountain bog is reported. Plants collected there include *Sarracenia purpurea* L., *Kalmia angustifolia* L., and *Cypripedium acaule* Ait. which, except possibly for the last species, previously were not known to occur in northern Georgia. The discovery of *Vaccinium hirsutum* Buckl. near Amicolola Falls 30 miles south of the N. Carolina-Georgia state line and of the blue-fruited *Crataegus brachyacantha* Engelm. and Sarg. in the southwest part of the state adds two species which are not reported by the manuals as occurring in Georgia. The altitudinal distribution of *Rhododendron catawbiense* Michx. in north Georgia is usually from 4,000 to 4,700 feet. However, in certain mountains in northwest Georgia it is found abundantly at an unusual elevation of 950 to 2,000 feet.

EPLING, CARL, University of California, Los Angeles, Calif. *The microgeographic distribution of Linanthus Parryae.*—*Linanthus Parryae* is a diminutive annual of the Mohave desert, an associate of the *Larrea* community. Depending upon the season, it fluctuates greatly in the size of its populations, growing in astronomical numbers in favorable years, almost disappearing from some localities in unfavorable years. Its position with reference to the other vegetation is fixed and characteristic. However great its abundance, it is restricted to the shallow depressions which lie between the larger perennials, around the base of which low mounds of sandy soil accumulate by wind action, thus, in favorable years, forming a widespread reticulum. In less favorable years this reticulum may be broken into a series of islands, which may in bad years disappear completely. The concentration of individuals varies within the reticulum, but the areas of greatest concentration remain in essentially the same position year after year. These areas form the islands of unfavorable years. Although the seeds are numerous, and it is impossible to believe that they are not carried upon the mounds, the limits of the population seldom transgress very high upon them. Hence, within this relatively uniform community it would appear that factors exist to restrict the establishment of individuals to an even more uniform and highly limited part of it. However widely the minute seeds might therefore be carried by wind or rain, the establishment of the species in a new environment and a different community would therefore seem highly improbable.

EPLING, CARL, AND HARLAN LEWIS., University of California, Los Angeles, Calif. *Fertility and natural hybridization in Delphinium and its bearing upon gene exchange and the origin of diploid species.* The degree of interfertility and the characteristics of natural hybridization in the genus *Delphinium* are considered in the light of their bearing upon the concept of "ecospecies" and the origin of certain wild diploid species. The following tentative conclusions are reached: 1. Interfertility between two species does not necessarily imply gene exchange, because of the intervention of other than sterility barriers, hence it does not necessarily imply subspecific status. 2. Gene exchange does not necessarily imply the modification of the entities exchanging them, because the channel for gene flow may be blocked by other barriers. 3. Under some conditions of the environment new combinations may become established and stabilized by means other than amphiploidy and evolve into new diploid species.

FLINT, LEWIS H., Louisiana State University, Baton Rouge. *Some freshwater red algae of Louisiana.*—Continued collections have revealed an unexpectedly rich flora surprisingly difficult to identify. Progress with the following plants of the southeastern section of the State is reported: *Porphyridium cruentum* Nägeli, *Batrachospermum vagum* (Roth.) Ag., *Batrachospermum macrosporum* Mont.,

Batrachospermum elegans Sirod., *Sirodotia suecica* Kylin, and *Compsopogon ludovicianum* n. sp.

FOSBERG, F. R., Plant Industry Station, United States Department of Agriculture, Beltsville, Md. *The immediate task of the herbarium.*—A consideration of the rapid and complete destruction of the temperate Andean flora of northern South America, due to population pressure and intense land utilization, destruction of Pacific Island floras by introduction of animals and by war activities, prospective destruction of mountaintop vegetation in the United States by radio station construction make it plain that if data on original vegetation and on the occurrence and distribution of rare plants are to be salvaged at all, this must be done immediately. The most urgent task before the herbaria at the present time is the carrying on of field work to make extensive collections with complete data from these interesting and rapidly disappearing floras.

GILLY, C. L., AND I. E. MELHUS, Iowa State College, Ames, Iowa. *Distribution and variability in teosinte.*—Teosinte occurs naturally in Guatemala and Mexico between latitudes 14° and 27° north, and from an elevation of about 600 to at least 2250 meters. It occurs, at present, as a series of isolated populations differing greatly in appearance and in the time required for maturity. The relative frequency of individuals in any one teosinte population, and the size of the area which it occupies, depend primarily on the environment and agriculture of that particular area. Plants of Guatemalan, Mexican and "Florida" teosinte grown at Ames, Iowa, have been compared in respect to growth habit and selected morphological characters. The Guatemalan plants had fewer floriferous nodes and fewer "ears" per ear-shoot than the others. The Mexican plants were the tallest and most corn-like in appearance, and had the longest basal internodes. The "Florida" plants were characterized by the greatest number of floriferous nodes with the lowermost ear-shoot developing much nearer the ground than in the other two types. A supplementary examination of available herbarium specimens reveals that geographical distribution and range of variability within this group of plants are considerably greater than is indicated in existing literature.

GOODMAN, GEORGE J., Department of Plant Sciences, University of Oklahoma, Norman, Okla. *Generic delimitation in the Eriogoneae.*—The logic of increasing the number of genera in the tribe to double the usually accepted number (8–10) or of reducing the number to 3, or even 1, is pointed out. Criteria accepted by taxonomists generally as important generic indicators in the group have been applied more consistently than heretofore through a more detailed study of the morphology. A technique for comparing the possible genera is outlined. The use of the method resulted in segregating slightly more genera than at present commonly accepted, but what is more important, the generic limits and relationships are delineated otherwise than before.

GRANT, MARTIN L., Iowa State Teachers College, Cedar Falls, Iowa. *Some vegetational features of Colombia.*—There are six extreme types of vegetation in Colombia, with an indefinite number of intermediate and transitional modifications: 1. Caribbean semi-desert, 2. Orinoco savanna, 3. Amazonian rain-forest, 4. Pacific rain-forest, 5. Temperate rain-forest, and 6. Alpine shrubby meadow (paramo). Results of a year's field work, mainly in the temperate rain-forest, are summarized. Illustrated with Kodachrome slides, and herbarium specimens of some of the more interesting types of plants encountered: fern (*Pteris*) fronds twenty feet long, *Equisetum* sixteen feet high, orchid inflorescences fifteen feet long, Balanophoraceous root-parasites, Rubiaceae (*Posoqueria*) with corollas a foot long, and composites (*Senecio*) forty-five feet high.

HAYDEN, ADA, Iowa State College. *The distribution of the remaining prairie areas in Iowa.*—During the past two summers a survey of the prairie areas remaining in Iowa has been made with reference to a program for state acquisition of tracts representative of the chief soil associations. The following data have been assembled for the areas examined: legal description, location with reference to soil types, description of vegetation, photographs, and specimens.

LEWIS, HARLAN, University of California, Los Angeles, Calif. *Leaf variation in Delphinium variegatum.*—In a colony of *Delphinium variegatum* T. & G. comparable leaves selected from the same individuals in different years may show a greater range in variability than is present in an entire colony in any given year. This non-genetic variation is discussed as a limitation to the use of representative or mass collections in studying the nature and range of variation of populations and as an advantage in using herbarium collections when adequate and suitable.

LEWIS, HARLAN, AND CARL EPLING, University of California, Los Angeles, Calif. *Formation of a diploid species of Delphinium by hybridization.*—*Delphinium recurvatum* Greene occurs on the saline flats in the San Joaquin Valley of California. In the oak woodland of the coastal mountains adjacent to the valley occurs *D. hesperium* Gray. A third species which occupies a region somewhat intermediate ecologically on the *Avena*-covered hills of the same valley is *D. gypsophilum* Evans. All three of these diploid species are equally constant and no intermediates or hybrids are known in nature. However, the morphology of *D. gypsophilum* is such that it suggests a combination of the characters of the other two entities. Although the cross *D. recurvatum* × *D. hesperium* is relatively sterile, F_1 hybrids are formed in the greenhouse and these are at least partially fertile when selfed, backcrossed to either parent, or crossed with *D. gypsophilum*. Among the hybrids we have found plants which are morphologically indistinguishable from *D. gypsophilum*. As yet we have no artificially produced true breeding strains so that the synthesis of *D. gypsophilum* is

not complete, but the evidence suggests to us that this has been its method of origin.

OWNBEY, MARION, AND HANNAH C. AASE, State College of Washington, Pullman, Wash. *Allium canadense and its allies in North America.*—An even dozen American species of *Allium* with fibrous-reticulate bulb coats and linear leaves form a natural alliance of which *A. canadense* is the historic type. The species are equally distributed into two subgroups, those which normally develop crests on the ovary, and those which never do. The species without crests are eastern in distribution; those with crests, with one exception, are western. Evidence points to the Southwest as the ancient center of dispersal for the entire alliance. Seven is the basic chromosome number in the six sexual species which have been examined cytologically, with tetraploid, $n = 14$, races also known in the four of these species which have been most intensively investigated. The tetraploids may or may not display quantitative or qualitative differences when grown with the corresponding diploids in a uniform environment. In nature, they appear to be ecologically or geographically separated. Two highly sterile tetraploid derivatives have become widespread by vegetative means and are treated as asexual species. Speciation appears to have occurred at the diploid level, with a subsequent doubling of the chromosome number in some of the species, the latter being accompanied or followed by the derivation of apomictic clones.

RICKETT, H. W., New York Botanical Garden, New York, N. Y. *Travels of Sessé and Mociño in New Spain.*—The *Archivo General de la Nacion* in Mexico contains much correspondence relating to the Botanical Expedition sent out by Charles II to survey the natural history of New Spain and to establish a botanical garden in Mexico. From these letters it has been possible to reconstruct in some detail the itineraries of Sessé and Mociño and their colleagues from 1788 to 1800. This information may be of use in evaluating the specimens which they collected.

STEYERMARK, JULIAN A., Chicago Natural History Museum, Chicago, Ill. *The Flora of Guatemala.*—The southern half of Guatemala forms part of the ancient land axis of Central America. The region of late Paleozoic and Mesozoic rocks has preserved the most ancient portion of the flora. Here occur genera which are considered as late Cretaceous and early Tertiary dispersal types. None of these genera are found in the more youthful areas of Guatemala. The extensive alpine regions of the Sierra de los Cuchumatanes, apparently because of their greater antiquity, as compared with the youthful summits of the volcanoes, have preserved most of the alpine flora. The cases of discontinuous distribution of identical species which occur in Guatemala and again in the highlands of South America indicate survivors of an earlier continuous land mass connecting Central and South America. The Mexican element is a conspicuous feature of the flora. Many of the species found in Guatemala, often at their southernmost limit of distribution, are identical or nearly identical with species of the United States. Some of them, such as *Berchemia scandens, Mitchella repens,* and *Parthenocissus quinquefolia,* reveal a definite relationship with the flora of the eastern United States, while another group has its affinities and origin with the flora of the Rocky Mountains or (and) southwestern United States. It is believed that the floral invasion of Guatemala began, following the withdrawal of Cretaceous seas, either at the end of the Cretaceous or at the beginning of the Tertiary period.

TRYON, R. M., JR., University of Minnesota, Minneapolis, Minn. *Spore characters of some North American species of Selaginella.*—The megaspores and microspores of species of *Selaginella,* subgenus *Euselaginella,* group of *S. rupestris* generally afford distinctive characters useful in their classification and identification. In size the megaspores range in different species from 260 micra to 560 micra and the microspores from 28 micra to 67 micra. The megaspores vary from smooth or nearly so to strongly and variously reticulate or tuberculate. They may or may not bear a crest. The microspores are smooth, striate, or reticulate; in some species there is a prominent wing. Similarities and differences in the spores are illustrated in the group of five species closely related to *S. rupestris.*

THE EFFECT OF IRON AND OTHER FACTORS ON THE PRODUCTION OF PIGMENT[1] BY THE YEAST TORULOPSIS PULCHERRIMA[2]

Catherine Roberts

FIRST DESCRIBED in 1887, the yeast *Torulopsis pulcherrima* (Lindner) Saccardo has since been the subject of numerous investigations which have often yielded conflicting results. Grosbüsch (1915) studied an isolate of this organism from apple peel and proposed for it the name *Torula rubefaciens* because of its striking red diffusible pigment. He concluded that the available oxygen, the kind and concentration of sugar, and the pH and duration of sterilization of the medium may influence pigment formation, but that light is without effect. Beijerinck (1918), presumably working with several isolates, reported that the fungus secretes a colorless chromogen which, under a sufficient supply of oxygen, reacts with iron present in the medium to produce the deep red pigment, which was believed to be closely related to the anthocyanins. He found that transfers from old cultures frequently gave rise to colorless colonies, which fact was explained by assuming that a transformation occurred in the cells of old cultures whereby some of them lost the ability to produce the colorless chromogen. At the same time, those cells which failed to undergo this modification tended to accumulate the pigment and die prematurely. The modified cells, on the other hand, were resistant to this toxic effect and consequently were the ones propagated, giving rise to non-pigmented subcultures. Punkari and Henrici (1933, 1935) studied cultural variation in Grosbüsch's isolate by means of single cell technique and found a marked dissociation of the colonies into red and white growth areas, the white type exhibiting a much greater stability than the red. Lodder (1934) and Castelli (1940) have reported that the presence of iron in the medium is necessary for pigment production, while Porchet (1938) has taken the opposite view. The present writer (1946), in investigating cultural variation among single cell progenies from a single isolate grown under relatively uniform environmental conditions, has confirmed Punkari and Henrici's findings on the characteristic instability of the red cultural type and the stability of the white variant. In rare instances red pigment appeared in white cultures which had remained non-pigmented for more than two years, making uncertain the interpretation of this variation as an expression of the irreversible dual phenomenon (Hansen, 1938).

[1] Since little is known of the chemical composition or biological function of the colored substance produced by *T. pulcherrima*, the convenient term "pigment" is used here in the broad biological sense of Fox (1944) to signify any colored substance in a living system, regardless of its biochemical significance.

[2] Received for publication November 19, 1945.

Since the work of Punkari and Henrici and the present author has shown that modifications of the genetic potentialities of individual cells occur regularly in at least two isolates when maintained under unvarying environmental conditions, the present paper deals with the stability of the various pigmented types of several isolates of *T. pulcherrima* when subjected to intentionally varied environmental factors.

MATERIALS AND METHODS.—Single cell cultures of each of the following ten isolates of *T. pulcherrima* were employed:

C-1.—Isolated from fermenting mushrooms (NRRL No. Y-412).

C-2.—Isolated from cider at Urbana, Illinois (NRRL No. Y-922).

C-4.—Isolated from cider at Urbana, Illinois (NRRL No. Y-741).

CV-4.—An originally non-pigmented variant from C-4, which had, however, produced a red pigment in culture prior to the present study.

C-5.—Isolated from fermenting blackberries at Peoria, Illinois.

C-7.—Isolated from cider at Urbana, Illinois (NRRL No. Y-749).

C-8.—Obtained from the American Type Culture Collection (No. 2514).

C-10a.—A pigmented type derived from Culture 214, which was originally isolated from California-grown Concord grapes.

C-10b.—A pigmented type also derived from Culture 214, but differing from C-10a in more abundant production of pigment.

CV-10.—A colorless variant originating as a white sector in a pigmented colony of Culture 214.[3]

The author is indebted to Dr. L. J. Wickerham of the Northern Regional Research I aboratory for the first six cultures listed above and to Dr. E. M. Mrak of the University of California for Culture 214.

In all of the tests reported in this paper the organisms were maintained on a standardized potato dextrose agar medium of the following composition: 250 g. potatoes, 20 g. dextrose, 20 g. agar, 1 liter tap water. The pH, when not adjusted, was within the range 5.8 to 6.5. Unless otherwise noted, all cultures were maintained at room temperature.

In studying the effect of oxygen on pigment production, anaerobic plates were employed. The plate consisted of a Pyrex glass dish 5 cm. in height and slightly over 9 cm. in diameter, the bottom of which was divided into two equal portions by a glass par-

[3] During the course of this study, the stock culture of the non-pigmented variant CV-10, chosen for its complete stability for more than two years, suddenly gave rise to a red pigment, the identity of which with the normal type of red pigment has not yet been established.

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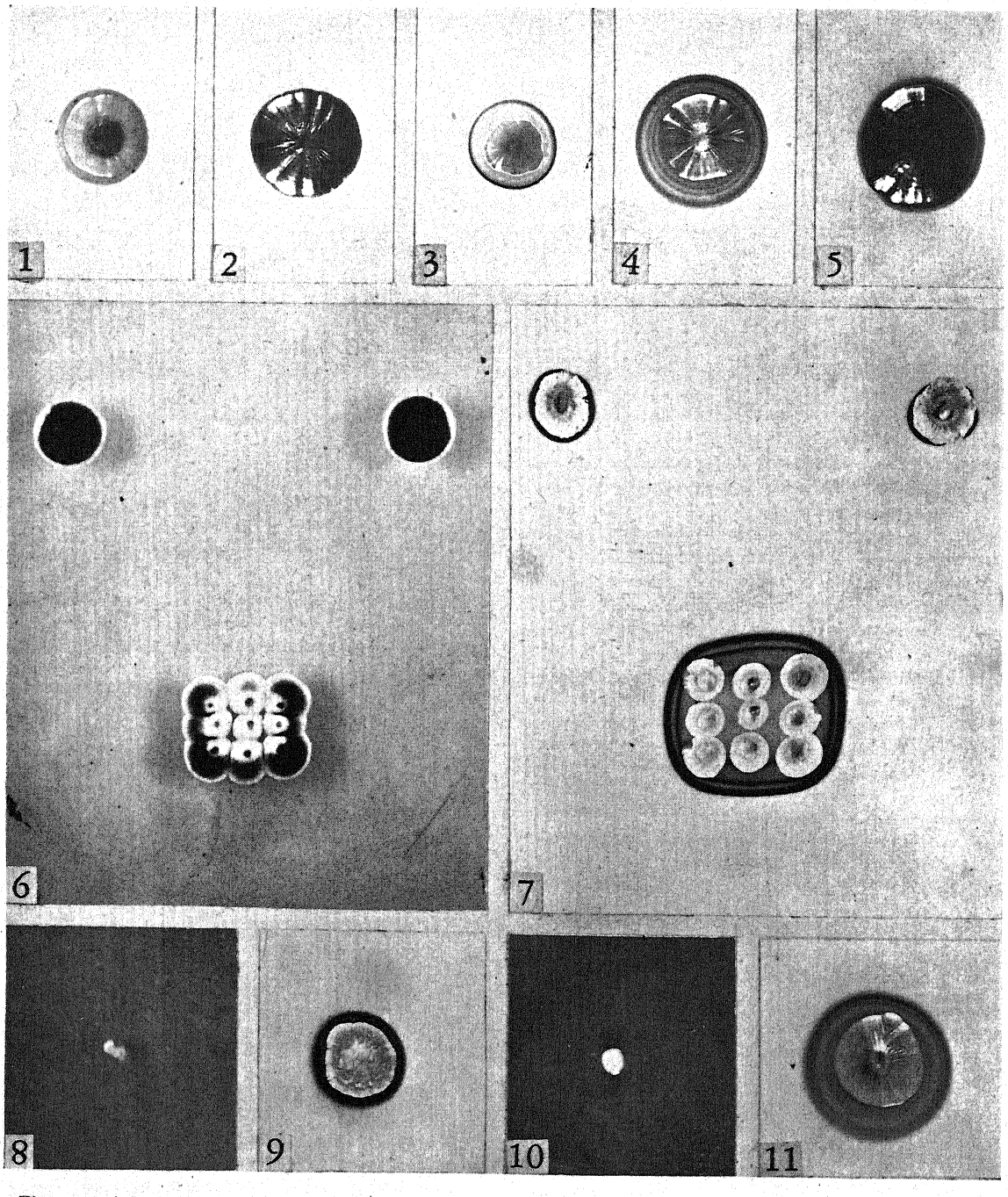


Fig. 1–11. Colonies of four isolates of *Torulopsis pulcherrima* showing effect of varying environmental factors on pigmentation and growth rate. ×1½.—Fig. 1–5. Effect of varying the amount of substratum.—Fig. 1. C-2, 5 cc. agar.—Fig. 2. C-2, 50 cc. agar.—Fig. 3. C-10b, 3 cc. agar.—Fig. 4. C-10b, 10 cc. agar.—Fig. 5. C-10b, 50 cc. agar.—Fig. 6–7. Effect of varying the position of the inocula.—Fig. 6. C-1.—Fig. 7. C-8.—Fig. 8–11. Effects produced after eight days under anaerobic and aerobic conditions.—Fig. 8. C-8, anaerobic (photographed over black paper).—Fig. 9. C-8, aerobic.—Fig. 10. C-10b, anaerobic (photographed over black paper).—Fig. 11. C-10b, aerobic.

tition. One gram of pyrogallic acid was placed in one portion of the dish and 20 cc. of 1N KOH in the other. After an inoculated poured plate was inverted over the top, as a lid, and the sides sealed with melted beeswax, the anaerobic dish was tilted several times to insure thorough mixing of the acid and base, resulting in absorption of the oxygen present in the chamber.

RESULTS AND DISCUSSION.—*The effect of the amount of substratum and the size and position of*

TABLE 1. *Pigment production as influenced by proximity of colonies.*

Isolate	Average diameter in mm.		Comparative intensity of pigment		Comparative amount of lateral diffusion	
	Isolated colonies	Adjacent colonies	Isolated colonies	Adjacent colonies	Isolated colonies	Adjacent colonies
C-1	7.0	4.0	+++	++	—	—
C-2	6.5	4.0	+++	++	—	—
C-4	7.0	4.5	++	+—	—	—
CV-4	7.5	4.5	+—	—	—	—
C-5	7.5	4.5	+++	++	—	—
C-7	7.5	4.0	+++	++	—	—
C-8	6.0	3.5	+	+—	+	+++
C-10a	7.0	5.0	+—	—	—	—
C-10b	8.0	5.0	+++	++	+	+++
CV-10	7.5	5.0	+—	—	—	—

the colonies.—Each of the 10 isolates was grown on the following amounts of substrata in Petri dishes: 3, 5, 10, 15, 20, 30, 40 and 50 cc. Each of the 80 plates was inoculated with a 2- or 4-day old culture by touching the tip of a needle bearing the inoculum to three equidistant points on the surface of the solidified agar. Recorded observations and photographs (fig. 1 to 5) were made after a 9- to 11-day incubation period. The pigmentation[4] of the three colonies in any one plate was essentially similar. The following observations were made: (1) In all isolates a progressive increase in colony size occurred as the amount of substratum increased, as would be expected on the basis of total nutrients available. (2) In all isolates except CV-10 an increasing intensity of pigmentation was correlated with increasing amounts of agar up to the point of maximum pigment intensity which ranged for the different isolates from 10 to 30 cc. per plate. (3) Five isolates produced a pigment capable of diffusing laterally into the surrounding medium, the maximum lateral diffusion occurring on 15 cc. or less of substratum, followed by a gradual decrease as the amount of agar increased. In C-8 and C-10b the diffusion occurred as well-defined concentric rings. The correlation found in this test between increased amounts of substrata and increased intensity of pigmentation is probably best explained on a purely nutritional basis, although it should be noted that the point of maximum intensity is presumably dependent upon inherent genetic factors, since it varies according to the isolate and is unchanged by further additions of nutrient substances. The measurements of the zones of lateral diffusion of pigment were not indicative of the total amounts of diffusion produced, since it was found in later tests that a considerable amount of vertical diffusion may also occur.

[4] All recorded observations on pigmentation were based upon the comparative intensity of color of the colonies. It is assumed that such macroscopic appearances coincided with the total amount of pigment produced, although the possibility exists that the coloration of the surface layer was not always indicative of the amount of pigment produced in the interior of the colony.

For studying the effect of varying the number of colonies maintained on a uniform depth and volume of substratum a series of ten different dilutions in sterile distilled water was prepared from a heavy suspension of cells from a culture of each of the ten isolates. One cc. from each of the 100 dilutions was spread over the surface of 20 cc. of solidified agar in a Petri dish, immediately poured off, and the plates incubated overnight in a tilted position, allowing excess water to collect at one edge. The next morning this water was poured off, and after three weeks, observations were recorded. It was found that all isolates, except CV-10, produced a diffusible pigment and that the width of the diffusion zone as well as the colonial diameter progressively increased as the number of colonies per plate decreased. In addition, a general tendency for intensified colonial pigmentation in proportion to increasing dilution of the suspension was noted.

In another test, each of the ten isolates was inoculated into a plate containing 20 cc. of agar. The needle bearing the inoculum touched the surface of the agar eleven times in such a manner that two inoculations were 35 to 40 mm. apart and the remaining nine were about 5 mm. apart. The results of this test, recorded after a seven-day incubation period, are given in table 1 and figures 6 and 7. It will be seen that with each isolate the diameter and pigment intensity of the isolated colonies were considerably greater than those of the grouped colonies, confirming the previous results in regard to the influence of nutritional factors on colonial size and pigmentation. It will be noted that the adjacent colonies of C-8 and C-10b showed much more lateral diffusion than did the isolated colonies. Figure 7, photographed seven days after inoculation, shows very clearly, however, that the nine adjacent colonies behaved as a single colony with respect to the concentric zones of diffused pigment. The size of this colony far exceeded that of the isolated colonies, and, since it had no competition for available nutrients, it was capable of producing a relatively large amount of pigment, most of which appeared to diffuse into the surrounding medium.

TABLE 2. *Pigmentation and diffusion of pigment occurring in stab cultures.*

Isolate	Color of surface colony	Color of stab	Vertical diffusion of pigment in mm.	
			After 9 days	After 6 weeks
C-1	Red	White	2.0	3.5
C-2	Red	White	3.0	4.0
C-4	Red	Red[a]	...	...
CV-4	Red	White	3.0	4.0
C-5	Red	White	1.5	4.0
C-7	Red	White	6.5	6.5
C-8	Red	White	12.0	12.0
C-10a	Red	White	1.0	1.0
C-10b	Red	White	9.0	9.0
CV-10	White	White	...	...

[a] The agar had cracked, allowing the growth below the surface to become exposed to the air and consequently resulted in pigment production along the exposed stab.

On the basis of these and the preceding results, it is evident that the production of pigment in *T. pulcherrima* is closely correlated with nutrition. Provided that the genetic factor for color is inherent in a given isolate, its full expression will largely depend upon the total amount of nutrients available to the individual colony. The maximum intensity of pigmentation is attained by well-isolated colonies growing on a relatively large volume of substratum.

The effect of light and temperature.—Three Petri dishes, each containing 20 cc. of agar, were streaked with a heavy suspension of cells from a two-day old culture of each of the ten isolates. One set of plates was placed in total darkness at room temperature, another was placed under a glass bell jar on a laboratory table, and the third was exposed to continuous light emanating from a Mazda Daylight 100-watt globe placed at a distance of two feet from the plates. After two weeks no differences whatsoever in amount of growth, pigment intensity, or pigment diffusion were detectable among the three plates of any one isolate. These results confirm Grosbüsch's findings on a single isolate.

Inoculated tubes of agar were placed at 13 different temperatures, ranging from 1° to 40°C., at 3° intervals. After five weeks it was found that the yeast grew at all temperatures except 40°C., the growth rate gradually falling off near the two extremes of the range. Optimum growth of all isolates occurred between 25° and 31°C. The highest intensity of color in the colony occurred between 7° and 13°C., while a marked decrease in intensity was apparent at 16°C. and at all higher temperatures. At the same time, little or no pigment diffusion occurred at the lower temperatures, while at 16° and continuing up to 28° or 31°C. much vertical diffusion occurred. Variation among the isolates in regard to the lowest temperature at which diffusion first occurred was evident, e.g., diffusion first appeared in C-7 at 7°C., in C-1 at 13°C., in CV-10 at 19°C., and in C-5 at 22°C.; in all isolates, however, with the exception of C-7, abundant diffusion occurred only at the higher temperatures and was accompanied by a decreasing intensity of colonial pigmentation. These results indicate that, although the total amount of pigment produced by *T. pulcherrima* in culture is probably not influenced by the temperature at which it is maintained, temperatures below 16° or 19°C. appear to favor colonial or cellular retention, and those above, to favor diffusion of the pigment.

The effect of oxygen.—During the course of several years' study of Culture 214, it had been noted that surface colonies produced more pigment than did submerged colonies. Several partially submerged colonies were found to develop pigment only where they projected above the agar surface, but when the submerged portion was cut into and exposed to the air, red pigment appeared on the colony in the region of the cut within 24 to 48 hours. This behaviour indicated that oxygen may be an important factor in pigment production, as had already been reported.

Dilution plates of all ten isolates were prepared and observations made twelve days after inoculation. Red pigment was found in the surface colonies of each isolate except CV-10. Although a red coloration was evident in the submerged colonies of all isolates except CV-4 and CV-10, it was, in general, paler than that of the surface colonies. All colonies at the bottom of the plates were completely non-pigmented except for those of C-2, in which a pale color was evident.

A series of stab cultures was prepared for each isolate and observations were recorded at the end of nine days and again at the end of six weeks (table 2). In addition to providing further evidence of the necessity for oxygen in the production of pigment, this test provided a rough method of comparing the relative amounts of pigment which the different isolates were capable of diffusing, since the diameter (but not the depth) of the colony, being limited by the diameter of the tube, was constant in all cases.

Six-day old cultures of all isolates were used as

inocula in the following test employing the previously described anaerobic chambers. Three inoculations of one isolate were made in each plate. An unsealed check plate containing 20 cc. of water in place of the pyrogallic acid-KOH mixture was prepared for each isolate. Observations were made after eight and nine days, when it was noted that each isolate except C-7 and CV-10 failed to develop any pigment under anaerobic conditions, while the checks, under aerobic conditions, exhibited normal pigmentation. Although the growth rate was markedly decreased under anaerobic conditions, this was not indicative of non-viability of the cells. On the afternoon of the eighth day after inoculation, observations were recorded and photographs taken (fig. 8 to 11), and, as a consequence, the wax seal on each anaerobic plate was broken, allowing air to enter the chamber. The following morning pigment had appeared over the entire surface of the colonies and the diameter of each had increased for all isolates except CV-10. The rapidity with which the pigment appeared supports the claim of Biejerinck that a colorless precursor of the pigment is elaborated by the cells, and that oxygen is necessary for its expression. The amount of pigment which appeared in colonies of C-7 and CV-10 under anaerobic conditions was extremely slight. Although no change in the amount of pigment occurred in CV-10 following the restoration of aerobic conditions, a considerable increase occurred in C-7, as in the other isolates.

The effect of iron concentration and acidity of the medium.—Preliminary experiments on iron concentration were carried out with additions of both ferric ammonium sulfate and ferrous ammonium sulfate to media until the following concentrations were obtained: 0.0001, 0.001, 0.01, 0.1, and 1.0 per cent iron salt. The pH was adjusted to 5.5 in all cases. In this and in succeeding tests 20 cc. of media were solidified in an upright tube and a single central inoculation made in each. After two weeks, similar results were obtained on both the ferrous and ferric salt media with each isolate: (1) The pigment intensity gradually increased with increasing concentrations up to 0.1 per cent (a concentration of 1.0 per cent did not readily support growth). (2) The vertical diffusion of pigment increased from 0.0001 per cent to 0.001 per cent iron salt concentration but fell rapidly at 0.01 per cent. Microscopic examination revealed that the pigment was retained within some of the individual cells when grown at an iron salt concentration of 0.01 per cent, but no such retention occurred at 0.001 per cent. Because of the fact that ferrous iron tends to become oxidized to ferric in a solution undergoing sterilization, it is believed that both the ferrous and the ferric ammonium sulfate media contained about the same proportions of Fe^{++} and Fe^{+++} in equilibrium, thus accounting for the similar results obtained. However, as Halvorson and Starkey (1927) have pointed out, only very small amounts of either ferrous or ferric iron will occur in solution at pH values above 5.0 due to precipitation of the iron as hydroxide. For this reason, ferric ammonium citrate was used as the source of iron in the remainder of the tests,[5] since Starkey and Halvorson (1927) have stated, "Iron may remain in solution in organic combination under conditions inhibitory to solution of inorganic iron, because of extremely low ionization of iron in organic compounds." It was believed that with this iron salt, more striking pigmentation should result because the organic iron would theoretically be available to the organism for a much longer period of time because of its greater stability in solution. After a two-week incubation period, it was found that the pigmentation behavior was essentially similar to that in the preceding test, including cellular retention of pigment at 0.01 per cent but not at 0.001 per cent iron salt concentration. A difference existed, however, in the relative amount of diffusion produced at various concentrations; in nearly all isolates, more diffusion occurred with organic iron than with inorganic at 0.0001 per cent and 0.001 per cent, and a greater intensity of colonial pigmentation was also noted in some isolates with organic iron. Both these differences could be reasonably correlated with the different degree of ionization of inorganic and organic iron and its consequent availability to the yeast. Comparative results obtained two weeks after inoculation with a single representative isolate are recorded in table 3.

Additional media were prepared containing organic iron salt at concentrations of 0.001, 0.0013, 0.002, 0.004, and 0.01 per cent in order to determine more definitely at what concentration diffusion is inhibited. The pH value was adjusted to 5.5, inoculations were made as before, and results recorded after three weeks. Again, the intensity of colonial pigmentation increased as did the concentration of the iron salt, and a progressive decrease in the amount of vertical diffusion was correlated with increasing iron salt concentration. However, the lowest concentration inhibiting diffusion was not identical for all isolates. CV-4, C-5, and C-10a first showed an absence of diffusion at a concentration of 0.002 per cent, C-4 and CV-10 at 0.004 per cent, C-1, C-2, C-7 (fig. 12), and C-8 at 0.01 per cent, while C-10b showed some diffusion at 0.01 per cent. As before, microscopic examination of some of the cultures revealed that at the iron concentrations inhibitory to diffusion cellular retention of pigment was noted, while cells from isolates exhibiting diffusion showed no, or only rare instances of, internal pigmentation. The fact that C-10b exhibited diffusion at a 0.01 per cent concentration in this test and not in the two preceding tests is believed to be explainable by the fact that observations were recorded three weeks after inoculation, rather than

[5] In a letter to the author dated June 26, 1944, similar results from unpublished tests on the effect on pigmentation in *Asporomyces uvae* of varying the concentration of organic and inorganic iron in yeast extract agar were obtained by Dr. L. J. Wickerham. *A. uvae* has been considered to be synonymous with *T. pulcherrima* by Mrak, Phaff, and Smith (1942).

TABLE 3. *The effect on pigmentation in Isolate C-10b of varying the concentration and source of iron in the medium.*

Source of iron	0.1% P	0.1% D	0.01% P	0.01% D	0.001% P	0.001% D	0.0001% P	0.0001% D	Without addition of iron P	Without addition of iron D
Ferric ammonium sulfate..	++++	0	+++	0	+	3.0	+--	3.0	+---	4.0
Ferric ammonium citrate..	++++	0	++++	0	+	11.0	+---	7.0		

P = comparative pigment intensity of colony.
D = vertical diffusion of pigment in mm.

two; that the time factor may influence the results was evidenced by reexamination of cultures seven weeks after inoculation, when the concentrations first inhibiting diffusion were found to be higher for several isolates.

Since Halvorson and Starkey (1927) found that as the acidity of a solution containing iron is decreased, there is a progressive decrease in the amounts of ferrous and ferric ions present, and since the above tests have indicated that a decrease in pigment intensity is correlated with a decrease in iron concentration, the following test of the effect of varying the pH under conditions of constant iron concentration was undertaken. No iron salt was added to the medium, the source of iron being that naturally occurring in potato tubers.[6] One and one-half liters of potato decoction were divided into four equal lots and the pH adjusted after addition of 2 per cent dextrose with 0.5 N HCl and 0.5 N NaOH, using a Beckman pH meter. After sterilization, the pH of each lot of agar medium was rechecked by the colorimetric method and yielded the following values: 4.0, 4.8, 5.2, and 6.2. After solidification in an upright position the 20 cc. of media in each tube were centrally inoculated with one of the ten isolates. Observations were recorded after two weeks, with the following results, of which table 4 is representative: (1) In each isolate the pigment of the colony decreased in intensity as the pH of the medium increased. (2) In each isolate the amount of vertical diffusion of pigment into the medium increased with increasing pH, the maximum amount being found either at pH 5.8 or 6.2. (3) The actual intensity of pigmentation in this test was in general less than that noted in the previous tests in which iron salt had been added to the medium. Thus, these results show that as the pH is increased, less ferric or ferrous iron is in solution, and therefore fewer ions are available to the organism, resulting in a decreased intensity of colonial pigmentation. Because diffusion of pigment increases with increasing pH, however, it is believed, as the previous tests on iron concentration have already indicated, that the concentration of ferrous

TABLE 4. *The effect of varying the pH of the medium on the pigmentation of Isolate C-4.*

pH medium	Pigment intensity	Vertical diffusion of pigment in mm.
4.0	+++	0
4.8	+	1.0
5.8	+—	1.5
6.2	—	2.0

[6] On the basis of Wehmer's figures (1931) for the percentage of Fe_2O_3 in the ash of potato tubers, it has been calculated that the amount of iron in potato dextrose agar may range from 0 to 0.012 per cent.

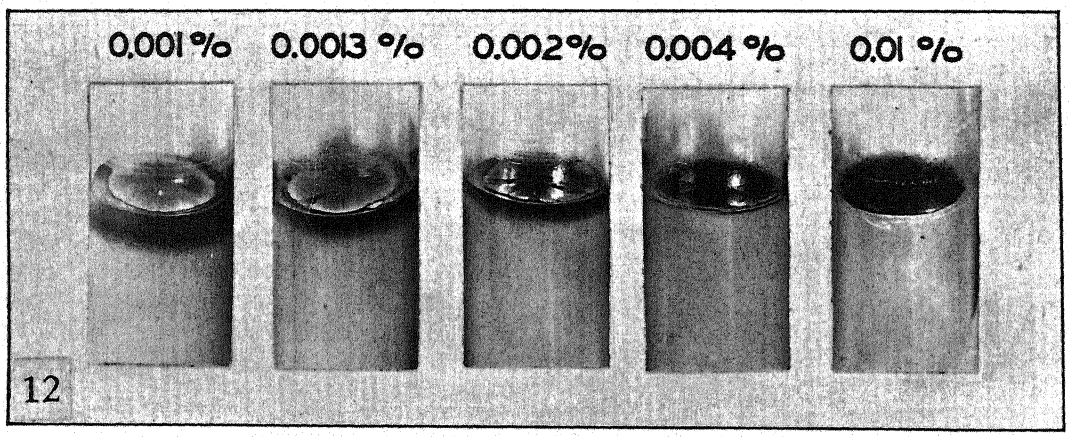


Fig. 12. The effect of increasing concentrations of ferric ammonium citrate on the pigmentation of Isolate C-7. Note increasing intensification of colonial color and decreasing pigment diffusion. Photographed three weeks after inoculation.

TABLE 5. *The effect of iron on pigment production under anaerobic and aerobic conditions.*

Isolate	Medium	Pigment intensity				Lateral diffusion of pigment in mm.			
		Under anaerobic conditions	Under aerobic conditions			Under anaerobic conditions	Under aerobic conditions		
			After 5½ hours	After 7 hours	After 24 hours		After 5½ hours	After 7 hours	After 24 hours
C-1	PDA	—	—	+---	+	0	Trace	Trace	0.5
C-1	PDA + Fe	—	+	++	+++	0	0	0	0
C-2	PDA	—	+—	+—	+	0	Trace	Trace	2.0
C-2	PDA + Fe	—	+	+++	+++++	0	0	0	0
C-7	PDA	—	—	—	+	0	0	Trace	0.8
C-7	PDA + Fe	—	+	++	++++	0	0	0	0

or ferric ions influences not so much the total amount of pigment produced as its retention or secretion by the individual cells.

Beijerinck (1918) postulated that a colorless precursor of the pigment is elaborated by the organism and under a sufficient supply of oxygen reacts with iron to produce the red pigment. In the present study, the experimental work on the effect of oxygen has indicated that such a precursor is formed. In order to determine whether this precursor is secreted into media of high or low iron salt concentration under anaerobic conditions, two lots of potato dextrose medium, pH 5.5, were prepared, one without the addition of any iron salt and the other containing 0.1 per cent ferric ammonium citrate. Anaerobic plates were employed, the same technique being followed as in previous tests. The cultures were maintained for two days under anaerobic conditions, after which aerobic conditions were restored. Table 5 is representative of the results secured from all isolates. It will be noted that there was a complete absence of pigmentation under anaerobic conditions, but within 5½ hours after restoration of aerobic conditions, pigment appeared in the colonies of those isolates growing on a high iron salt medium, and in the medium surrounding those colonies growing on a substratum low in iron. It will further be seen that after 24 hours intense colonial pigmentation without diffusion occurred in the high iron medium, and low colonial pigmentation accompanied by abundant diffusion occurred where the medium was of a relatively low iron content. It is believed that the rapidity with which the color appeared upon restoration of oxygen again provides evidence in support of Beijerinck's statements regarding the existence of a precursor of the pigment.

A plausible explanation of the diffusion behavior was suggested by Beijerinck, i.e., under low concentrations of iron the ferrous or ferric ions in the vicinity of the cells are insufficient to combine with all of the precursor elaborated, and consequently, it diffuses into the medium and unites there with the iron; in a substratum of high iron content, the ferrous or ferric ions enter the cells and unite there with the colorless chromogen. Such an explanation, however, is speculative, since experimental proof of the movements of the ions has not been obtained.

Since the white variant CV-10 was found to be unstable by the formation of pigment, information on the elaboration of the precursor in a stable white variant was desirable. Accordingly, on the surface of a stable white, five-day old colony, originally derived from a single cell from a sector of a pigmented colony, a small crystal of ferric ammonium citrate was placed. No pigment ever appeared in the colony or medium, whereas when iron salt was similarly added to a red colony, a very deep color appeared within twelve hours. This test was repeated several times with similar results, and in one case the beginning of intensification of color appeared in a red colony within 80 minutes after addition of the iron salt. This evidence confirms Beijerinck's statement that the stable, non-pigmented variants of *T. pulcherrima* have lost the ability to elaborate a pigment precursor.

Through using mass transfer technique, Beijerinck concluded that individual cells which accumulated the pigment died prematurely. This was tested by single cell technique. From a heavily pigmented culture of C-7 growing on a medium containing 0.1 per cent ferric ammonium citrate a number of single cells were isolated, half of which were completely colorless and half with accumulated red pigment. The percentage of germination of the hyaline cells was 70 per cent, while that of the red cells was zero. Red cells from other cultures were isolated at various times and maintained under the most favorable conditions without budding ever occurring. This evidence, together with the poor growth obtained on media containing 1 per cent ferric ammonium citrate, substantiates the following statement of Beijerinck: ". . . . à concentration élevèe le pigment, donc le fer, devient un poison pour les cellules non transformées. . . ."

SUMMARY

Ten different single cell isolates of *Torulopsis pulcherrima,* varying in the amount of red pigment produced when maintained under uniform laboratory conditions, were subjected to alterations of environmental factors to determine how such changes influence pigment production.

Nine isolates reacted in a similar way to all of the environmental factors tested. One pure white variant, which had remained stable in culture for

more than two years, showed no change during several of the tests. It then suddenly gave rise to a red pigment and in the subsequent tests reacted toward environmental changes as did the other isolates.

A relationship between pigmentation and the total amount of nutrient substances available to the colony was found.

Light was without effect on pigmentation.

Although the total amount of pigment formed in each isolate did not appear to be influenced by the temperatures at which it was maintained, temperatures below 16° or 19°C. appeared to favor retention of the pigment within the colony, while higher temperatures permitted the pigment to diffuse into the medium.

Increasing concentrations of ferric ammonium citrate added to the basal medium were correlated with an increase in intensity of colonial pigmentation up to a concentration of 0.1 per cent. Diffusion of the pigment increased with an increase in iron salt concentration up to 0.001 per cent but fell rapidly at 0.01 per cent. At concentrations inhibitory to diffusion the pigment was retained within many of the individual cells, while only rare instances of cellular retention occurred at concentrations allowing diffusion to occur.

As the pH of the medium increased from 4.8 to 6.2, the pigment intensity of the colonies decreased while diffusion of the pigment increased.

In the absence of oxygen, the formation of pigment was inhibited. Within 5½ hours after restoration of aerobic conditions, pigment appeared.

Evidence was obtained which substantiates Beijerinck's claim that individual cells which accumulate the pigment are incapable of further growth, and that the stable white variants of the organism have lost the ability to elaborate the precursor of the pigment.

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THE VITAMIN REQUIREMENTS OF MEMNONIELLA AND STACHYBOTRYS [1]

Paul B. Marsh and Katharina Bollenbacher[2]

MEMNONIELLA ECHINATA (Riv.) Galloway (1933) (fig. 1) has been mentioned in numerous unpublished reports from Australia as one of the cellulose-decomposing fungi most commonly occurring on and causing damage to tents, tarpaulins, and other items made of cotton fabric and exposed to the weather in humid tropical areas. Consequent interest in the growth requirements of this fungus led to the experiments herein described. Six isolates of *Memnoniella* and five isolates from the very closely related genus *Stachybotrys* have been studied. Each of these isolates has been found to require the vitamin biotin for its growth. Experiments with fifteen other vitamins and related materials, however, have failed to reveal the existence of any other growth factors for these fungi. A glucose-mineral salts-biotin liquid medium has been observed to be sufficient not only for mycelial growth but also for abundant sporulation, provided that a glass cloth wick or other suitable physical substratum for sporulation is present. A brown sugar-mineral salts-agar medium is suggested as convenient for routine culture work and excellent for producing heavily-sporulating cultures. In laboratory tests both *Memnoniella* and *Stachybotrys* grow readily on, and cause rapid loss in strength of, grey cotton duck, which is thus apparently an adequate source of biotin for their nutrition.

MATERIALS AND METHODS.—During the summer of 1944 several cultures of *Memnoniella* and *Stachybotrys* were sent to us upon our request from the NDRC Tropical Fungus Culture Collection at Harvard. These cultures were made available to us by Dr. Wm. H. Weston who had obtained them through the courtesy of the Mycological Panel of the Australian Scientific Liaison Bureau. The cultures had been isolated originally from deteriorated material from the Southwest Pacific. Cultures from Prof. Weston are identified in the tables by the designation "SN" preceding the isolate number. Other isolations were made in this laboratory from damaged cotton fabric of local origin.

The vitamin and accessory growth substance preparations used were: biotin, obtained as ampoules containing 25 gammas crystalline biotin in 1 cc. of distilled water (SMACO); vitamin B_1, crystalline thiamine hydrochloride (Merck); vitamin B_6, crystalline pyridoxine (Merck); vitamin C, crystalline ascorbic acid (Merck); riboflavin, U. S. P. (Merck); pimelic acid (Eastman Kodak); calcium pantothenate, dextrorotatory (Merck); and inositol (Merck). Dr. R. A. Steinberg kindly provided us with folic acid (a preparation originally obtained from Dr. R. J. Williams), beta alanine (Eastman Kodak), 2-methyl-1, 4-naphthoquinone (Eastman Kodak), nicotinic acid (Eastman Kodak), hemin (Eastman Kodak), alpha tocopherol (Eastman Kodak), p-amino benzoic acid (Eastman Kodak), and glycollic acid (Eastman Kodak). All salts and the dextrose used were of CP grade. Cotton extract was prepared by kneading 50 g. of raw cotton with 500 cc. of distilled water. One cc. of a 1–10 dilution of this extract was used per flask as the cotton extract supplement recorded in table 1.

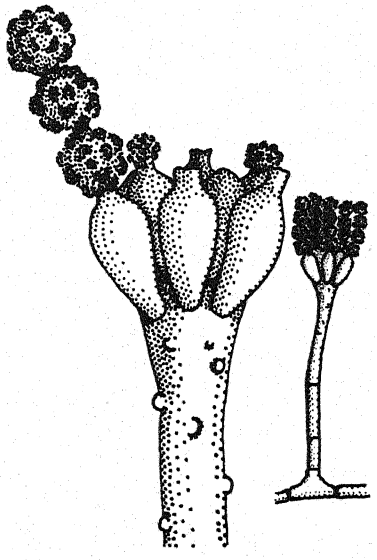


Fig. 1. Phialophore, phialides, and spores of *Memnoniella* (drawing made by Seth Pope).

The culture vessels used for the vitamin experiments were 250 cc. pyrex Erlenmeyer flasks, cleaned before the experiments in dichromate-sulphuric acid cleaning solution. Each flask contained in all cases a total of 25 cc. of liquid medium, sterilized at approximately 15 pounds steam pressure for 15 minutes. Inoculation was carried out by carefully picking off spores with a sterile needle from the surface of cultures growing in tubes on cotton extract-glucose-mineral salts agar, and transferring them to the liquid in the experimental flasks. After incubation for several days at approximately 30°C., the cultures were filtered through sintered glass weighing crucibles, and the mycelium washed with about 15 cc. of water, dried in an oven overnight at 100°C., cooled in a desiccator, and weighed. Shaking-culture incubations were carried out at 28°C. on a laboratory

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TABLE 1. *Effect of biotin and vitamins B_1 and B_6 on the growth of isolates of Memnoniella and Stachybotrys as shown by dry weight in milligrams of fungus growth in liquid culture. The basal medium contained 1 per cent glucose plus millimolar concentrations of $NaNO_3$, KH_2PO_4, K_2HPO_4, and $MgSO_4$-$7H_2O$ of 10, 5, 5, and 1, respectively (i.e., 0.85, 0.68, 0.87, and 0.25 g. per liter), plus .1 ppm Fe and Zn as sulphate and chloride respectively, and 0.025 ppm Mn as sulphate.*

Fungus	Days incubation	Gammas vitamin per flask: Biotin .0001	Biotin .0003	Biotin .001	Biotin .003	Biotin .01	Biotin .1	B_1 100	B_6 100	Cotton extract	No vitamin
Memnoniella SN 2	8	2	2	6	3	24	59	1	0	12	1, 0
Memnoniella SN 52	8	1	2	0	2	7	26	2	0	8	0, 0
Memnoniella SN 85	8	1	2	1	3	7	39	0	1	12	0, 6
Memnoniella SN 86	13	1	1	9	15	30	42	0	0	10	0, 0
Memnoniella 64.1	13	0	1	8	15	23	38	0	0	8	0, 0
Memnoniella 1229.2	8	1	1	4	7	10	33	0	1	9	0, 0
Stachybotrys SN 1	8	0	2	21	29	34	39	0	..	7	0, 0
Stachybotrys SN 65	8	0	2	14	19	23	27	0	1	14	1, 1
Stachybotrys SN 70	8	0	1	4	22	21	27	0	3	16	0, 0
Stachybotrys 7	8	11	13	15	58	50	46	8	0	48	1, 7
Stachybotrys 35	8	0	1	18	58	59	49	1	0	69	0, 0

shaker having a horizontal motion of 1¼ inches with 95 complete cycles per minute.

EXPERIMENTAL RESULTS.—Table 1 shows the dry weights of the mycelial growths of several *Stachybotrys* and *Memnoniella* isolates after incubation on a glucose-mineral salts medium with and without added vitamins. Distinctly significant growth responses are seen with a majority of the isolates in cultures containing as little as 0.001 gamma of biotin per flask. Of the eleven isolates tested, all required added biotin for their growth. While this fact does not, of course, indicate that all or even most strains of fungi in these two genera would behave similarly, it does seem to suggest that biotin deficiencies are very common among them. Vitamins B_1 and B_6, applied separately over a wide range of concentrations, failed to support growth in the absence of biotin. Results with flasks containing very high concentrations of these vitamins are recorded in table 1. Relatively small amounts of an aqueous extract of

TABLE 2. *Growth of Memnoniella echinata 1229.2 and Stachybotrys SN 70 on a basal glucose salts solution (see table 1) containing additional growth factors in the presence and absence of biotin.*

Growth factors, amount per flask	Milligrams dry weight of mycelium: *Memnoniella* 1229.2 Biotin absent	*Memnoniella* 1229.2 Biotin present, 0.1 gamma per flask	*Stachybotrys* SN 70 Biotin absent	*Stachybotrys* SN 70 Biotin present, 0.1 gamma per flask
Experiment 1, shaking cultures, 12 days, 28°C.:				
Ascorbic acid, 12 gammas	0.3	101	1.0	52
Calcium pantothenate, 6 gammas	0.6	104	0.5	57
Inositol, 1 mg	0.3	95	0	55
Pimelic acid, 6 gammas	0.3	101	0	52
Riboflavin, 3.8 gammas	0	102	0	57
Vitamin B_1, 100 gammas	...	...	0	55
Vitamin B_6, 100 gammas	...	...	0	55
None	0.2	95	0	55, 58
None	...	96	...	54, 50
None	...	111	...	55, 53
Experiment 2, static cultures, 12 days, 30°C.:				
Alpha tocopherol, 1 mg	0	44	...	.....
Beta alanine, .05 mg	0.2	41	...	.....
Folic acid, 5 gammas (total solids)	0	44	...	.....
Glycollic acid, 0.5 mg	0.2	49	...	.....
Hemin, .05 mg	0	51	...	.....
Nicotinic acid, .05 mg	0	46	...	.....
p-amino benzoic acid, .05 mg	0	46	...	.....
2-methyl-1,4-naphthoquinone .05 mg	0	34	...	.....
None	0	43	...	.....

raw cotton, however, permitted good growth. Since several different samples of cotton were used and since there was no attempt to obtain complete extraction of biotin in preparing the cotton extract, the growth figures in table 1 cannot be considered to furnish more than a rough approximation of the biotin content of cotton. Robbins and Ma (1942a) have reported the presence of biotin and vitamins B_1 and B_6 in cotton batting.

Prior to the experiments shown in table 1, attempts to demonstrate the influence of biotin on growth in a medium similar to the medium of table 1 but containing ammonium nitrate instead of sodium nitrate as a source of nitrogen and 2 per cent of glucose had been successful with each of the six *Memnoniella* isolates tested. The maximum total growth with the highest biotin concentration of 0.1 gamma per flask, however, was only about 15 mg., apparently a result of the fact that the medium became rapidly and rather strongly acid under these conditions.

Table 2 presents the results of two experiments in which growth measurements were made after the fungi had been incubated in flasks containing several growth factors other than biotin in the presence and absence of the latter. None of these additional materials appeared to influence growth. The ineffectiveness of pimelic acid parallels the results obtained by Robbins and Ma (1942b) with eight species of *Ceratostomella, Grosmannia serpens, Fusarium avenaceum, Neurospora sitophila, N. tetraspora,* and *Ashbya gossypii.* The ineffectiveness of vitamins other than biotin was further demonstrated in an

TABLE 3. *Ineffectiveness of a combination of eight other growth factors on the growth of Memnoniella 1229.2 in the presence and absence of biotin.*

Biotin per flask	Milligrams dry wt. of mycelium with vitamin combination	
	Absent	Present
None	0	0.1
0.01 gamma	30	33
0.1 gamma	61	64

In terms of vitamin content per flask the combined vitamin solution contained:

100 gammas vitamin B_1, 100 gammas vitamin B_6, 12 gammas ascorbic acid, 6 gammas pimelic acid, 6 gammas calcium pantothenate, 3.8 gammas riboflavin, 1 mg. inositol, and 50 gammas nicotinic acid.

The basal glucose salts solution is described in table 1. Cultures incubated eight days at 30°C.

experiment in which *Memnoniella* was grown in liquid culture in the presence and absence of a combination of eight vitamins with and without biotin. As may be seen in table 3, the combined mixture of vitamins had no influence on growth either in the presence or absence of biotin.

In the experiments recorded in table 1 and 2, the fungus mycelium was almost entirely submerged and sporulation occurred only on a few isolated bits of floating mycelium. The amount of sporulation was always small but was not uniform from one experiment to the next and apparently depended merely on the extent to which small fragments of mycelium floated at the surface. When, however, a piece of glass cloth, previously cleaned successively in toluene and in dichromate-sulphuric acid cleaning solution was placed as a wick into a glucose-mineral salts-biotin solution of the same glucose and salts composition as recorded in table 1 but containing 1

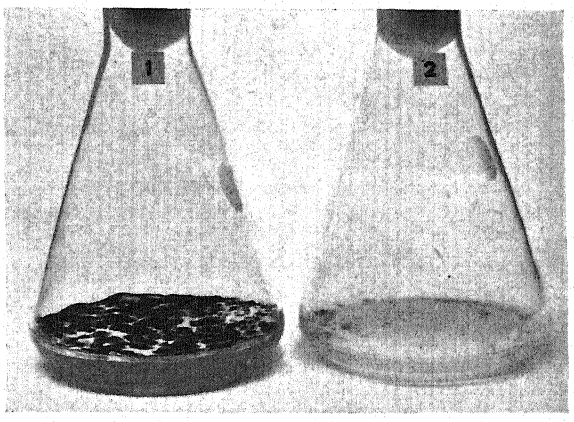


Fig. 2. Sporulation of *Memnoniella* 1229.2 on liquid medium containing mineral salts, filter paper, and 1 gamma of biotin per flask. Flask 1, filter paper elevated on glass beads. Flask 2, control, filter paper submerged.

gamma biotin per flask, the fungus grew rapidly on the wick and sporulated abundantly. A control flask in which the same glass cloth was submerged beneath the water level showed only very slight sporulation. It was apparent that the fungus did not require any additional growth factors other than biotin for sporulation but only a suitable physical substratum. Presumably an adequate supply of oxygen is necessary for sporulation and this is secured by the use of a suitable substratum. If the fungus was cultured on a filter paper disc resting on the surface of glass beads in an Erlenmeyer flask containing mineral salts and biotin, abundant sporulation was obtained (fig. 2). The filter paper in this case must, of course, be wetted by the solution but not submerged. It serves as a source of carbon and may also provide a suitable physical substratum for sporulation. In a mineral salts-biotin liquid culture containing a submerged filter paper disc, mycelial growth takes place, but there is very little sporulation.

There are undoubtedly many natural sources of biotin which could be used for routine cultures of *Memnoniella* and *Stachybotrys* in which it is not desired to use synthetic biotin. Brown sugar has served very satisfactorily for this purpose in our own work in a medium containing 0.24, 0.22, 0.16, and 0.18 g. per liter of NH_4NO_3, KH_2PO_4, K_2HPO_4, and $MgSO_4$-$7H_2O$, respectively, in addition to 20 g. of agar and 5 g. of brown sugar. A 2

per cent agar containing 0.5 per cent glucose, 0.1 per cent yeast extract and salts, as described for the brown sugar medium just mentioned, gave good growth and sporulation. A Difco corn meal extract agar preparation appeared to be excellent for inducing sporulation although not adapted to producing rapid mycelial growth. When inoculating on this medium, it was found desirable to cover the surface of the agar thoroughly with spores from a liquid suspension, and under these conditions excellent sporulating cultures were obtained. This corn meal medium as made up was stated to contain the following quantities of materials per liter: infusion from 50 g. of corn meal, 2 g. dextrose, and 15 g. agar. In general, with a 30°C. incubation temperature, heavy sporulation was obtained on any of the three media just described in seven days.

TABLE 4. *Per cent strength loss of 8-ounce grey cotton duck after spore-dusting inoculation with Memnoniella or Stachybotrys and four-week incubation at high humidity and 30°C. Original fabric strength, "Q" duck—119 lbs., "M-2" duck—123 lbs.*

Fungus	Per cent strength loss	
	"Q" duck	"M-2" duck
Memnoniella 64	62	55
Memnoniella SN 85	87	60
Memnoniella 1229.2	67	62
Stachybotrys 7	44	37
Stachybotrys SN 65	55	48

While no estimates of the actual amount of biotin present in grey cotton duck have been made, the biotin-deficient isolates of *Memnoniella* and *Stachybotrys* encountered in this study grow readily on it. In experiments following the principle of an isolation technique described by Zuck (1946), it has been found that strips of grey duck dusted with spores of *Memnoniella* or *Stachybotrys* and incubated at high humidity decrease rapidly in strength as the characteristic black sporulating growth of the fungus develops. In these experiments (table 4) strips of an 8-ounce duck cut 6″ × 1½″ with the long dimension parallel to the warp and ravelled to exactly one inch in width were hung on hooks attached to the tops of 500-cc. bottles containing a small amount of water in the bottom. The bottles, containing the strips, were sterilized in an upright position in an autoclave at 15 pounds steam pressure for 15 minutes. The strips were inoculated by dusting on spores of the fungi with a sterile brush and were then incubated at 30°C. for four weeks. The strips were then washed, dried, conditioned over a saturated sodium nitrite solution in a desiccator, and broken on a Scott tester.

Figure 3 shows one uninoculated and two inoculated and incubated strips of cotton duck. The strip at the left of the photograph was inoculated by dusting on spores and was incubated for two weeks at high humidity as described above, whereas the strip in the center, showing very heavy sporulation, was wet in water, sterilized, placed on mineral salts agar, inoculated by pipette with 1 cc. of spore suspension and incubated at 30°C. for two weeks.

DISCUSSION.—Workers in the field of mildew deterioration of fabrics have long been acquainted with a black-spored fungus having the prominent phialides characteristic of the genus *Stachybotrys*. Thom, Humfeld, and Holman (1934) included *Stachybotrys* among a group of other fungi in their culture bottle tests on fabric tendering. Early experience with isolates of *Stachybotrys* in the writers' laboratory, however, was disappointing in that growth was usually very poor on the filter paper-mineral salts-agar media which had been used with such excellent success for other cellulose-decomposing fungi (Marsh *et al.*, 1945). It was unexpected

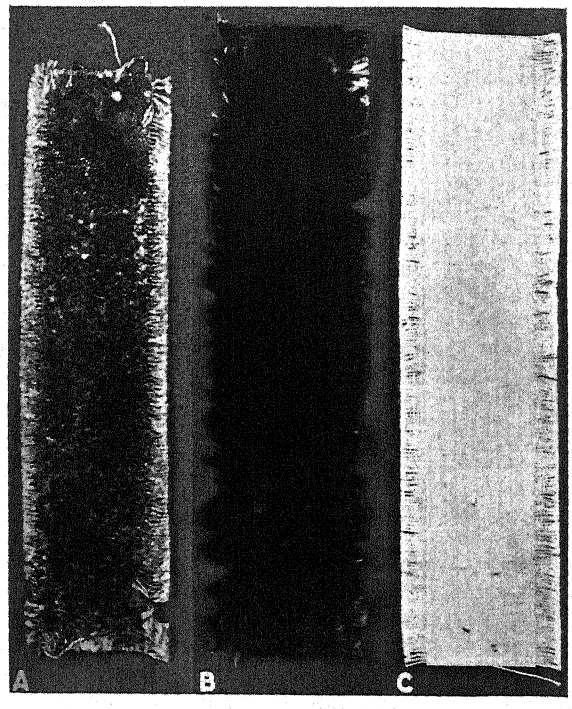


Fig. 3. Strips of cotton duck with growths of *Memnoniella*. A: Inoculation by spore-dusting and incubation in humid atmosphere. B: Pipette inoculation of spore suspension and incubation on mineral salts agar. C: Uninoculated strip.

and interesting, therefore, when reports came from Australia to the effect that both *Stachybotrys* and a closely related fungus called *Memnoniella echinata* were being found very frequently to be a cause of rotting of tents, tarpaulins, and other cotton fabric articles; certain isolates of *Memnoniella* were stated to be finding quite general use as mildew-resistance specification test organisms in the procurement of military fabrics. The new combination *Memnoniella echinata* had been made by Galloway (1933) to describe a fungus obviously closely related to *Stachybotrys, with Stachybotrys*-like phialides but with

spherical spores in chains rather than elliptical or subglobose spores in heads as had been previously described for *Stachybotrys*. Zuck (1946) has recently pointed out that certain isolates are actually intermediate between *Stachybotrys* and *Memnoniella* in that they possess both elliptical spores in heads and spherical spores in chains.

It occurred to the writers that despite their unfavorable early experience with *Stachybotrys*, they might find the Australian *Memnoniella* and *Stachybotrys* isolates to have some special merits as fabric mildew resistance test organisms. Several cultures of the two fungi obtained from Australia, however, grew only very poorly on filter paper-mineral salts-agar media, exactly as had the previous *Stachybotrys* isolates obtained in this country. A vitamin deficiency was suspected and the experiments herein described were carried out. In connection with their fabric tests, the Australians had apparently been growing their stock cultures on some natural medium without specifically recognizing any vitamin requirement by the fungi. A recent paper by Drobotko (1945) raises a question concerning the advisability of using *Stachybotrys* or *Memnoniella* in routine laboratory tests, inasmuch as *S. alternans* on moldy hay was shown to be the causal agent of a disease in horses. The organism was suspected to be pathogenic to man as well, although no conclusive evidence was offered on the latter point.

Since so many different types of fungi decompose cellulose and tender cotton fabric under at least some conditions of natural exposure (Thaysen and Bunker, 1927) and since the vitamin requirements of fungi differ widely (Robbins and Kavanagh, 1942; Schopfer, 1943), it is difficult to judge without further evidence just how important the vitamin content of cotton fabric may be in the general problem of its resistance to mildewing and rotting. In England, Galloway and Burgess (1940) and later Fargher (1945) have stressed the importance of scouring cotton thoroughly if it is to serve under conditions conducive to mildew. Scouring removes the mineral salts and nitrogen essential to fungus growth. The water-soluble B complex vitamins, B_1 and B_6, and biotin are also in all probability largely removed. These vitamins are known to be required by many fungi (Robbins and Kavanagh, 1942; Schopfer, 1943). While the use of highly purified cotton fabrics would in all probability be of little avail as a protective measure for cotton to serve in contact with soils, it seems probable to the writers that for certain above-ground uses a practical measure of protection might be secured by this means.

SUMMARY

Experiments have been carried out on the vitamin requirements of six isolates of the cellulose-decomposing fungus *Memnoniella*, and five isolates from the closely related genus *Stachybotrys*.

All of the isolates studied require biotin for their growth. The amounts of biotin required are very low, of the same order of magnitude as has been found effective by previous workers using several other micro-organisms.

Experiments with 15 other vitamins and similar materials have failed to reveal any influence of these substances on growth.

Abundant sporulation occurs on a synthetic liquid medium containing biotin as the only vitamin, provided a suitable physical substratum for sporulating growth is used.

A medium containing mineral salts, agar, and brown sugar has been found satisfactory in routine culture procedures to obtain good growth and sporulation. Yeast extract and corn meal agar media have also been found satisfactory.

Memnoniella and *Stachybotrys* grow readily on unbleached cotton duck, indicating the presence of biotin in the fabric.

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THE DEVELOPMENT OF STERILE OVULES IN LYCOPERSICON ESCULENTUM MILL.[1]

Charles M. Rick

PLANTS THAT are unfruitful for cytogenetic reasons appear in field populations of horticultural tomatoes (*Lycopersicon esculentum* Mill.) at the rate of about one per 1,000 fruitful plants. Abortion of pollen or ovules largely explains the barren condition of these plants. The gametic sterility, in turn, may reflect a breakdown in development of the gametophyte—a breakdown caused by recessive genes in diploids or by unbalanced chromosomal complements in heteroploids. The causes of sterility in a random sample of unfruitful plants were recently investigated (Rick, 1945).

In the cytogenetic survey made, functionless ovules were found to consist of two strikingly different types, whose development is described in the present report.

Thin sections of ovaries at anthesis or at earlier developmental stages were fixed in form-acetic-alcohol (formula used by Cooper, 1931). After imbedding in paraffin and sectioning, the material was stained in iron-alum haematoxylin according to Esau's (1944) schedule. Ovaries fixed at anthesis provide the data in table 1.

Except where noted otherwise, ovaries were collected in the late summer from plants growing in the field at Davis. A very high proportion of normal ovules was characteristic of ovaries collected from fruitful plants at this time. That conditions were also favorable for fruiting is indicated by the fact that unfruitfulness was observed only in plants that were cytogenetically sterile.

Appearance of fertile and sterile ovules at anthesis.—A fertile tomato ovule contains an embryo sac that is highly vacuolate and greatly distended. The embryo sac is broadly ellipsoidal, its dimensions in these preparations averaging 41 $\times$63 μ. The same features are found in freehand sections of living ovules. Since the nuclei of the embryo sac are distributed in various planes throughout this large cavity, it is seldom possible to find all nuclei in a single microtome section. Despite this enlarged condition, one can readily identify normal embryo sacs by scanning serial sections. The synergid cells and polar nuclei are shown in one plane in figure 6.

The synergid cells are characteristically cuneate in shape. At anthesis the nucleus appears at the narrow micropylar end of the synergid cell, while a single vacuole occupies the other broadened end. The egg cell is usually in contact with the synergids, often surmounting their vacuolate ends. The polar nuclei, which at anthesis may exist in separate (fig. 6) or fused condition, usually appear midway between the micropylar and chalazal ends of the embryo sac and are found mostly in the peripheral cytoplasmic layer. Occasionally, however, they may be observed in the center of the large vacuole, connected with the periphery by thin cytoplasmic strands. The three antipodal cells disintegrate during the later stages of maturation; as a rule, only vestiges of them remain at anthesis. The nucellar epidermis, which collapses earlier in development, is recognized at anthesis only by a thin layer of crushed cell walls immediately surrounding the embryo sac. The remains of the nucellar epidermis are surrounded by densely cytoplasmic, parenchymatous, integumentary cells, roughly isodiametric in shape. The main body of the ovule consists of similar, though more vacuolate, cells. The single integument is penetrated by a micropyle, whose length averages 125 μ.

The sterile types contrast sharply in appearance with normal ovules. In one, arbitrarily called the *collapsed* type (fig. 11, 12, 20), the embryo sac cavity is variously expanded at anthesis, but is never so large as in normal ovules. Ovules of the collapsed type can be identified easily by this difference in size of the embryo sac cavity. Instead of the structures described above, small, dark-staining, irregularly shaped masses occupy the cavity. These contents—judging from their degenerate and amorphic condition—are probably the remains of gametophytes that collapsed before maturity. Studies of development bear out this assumption. A nucellar epidermis in various stages of collapse can always be distinguished.

Occasionally an ovule at anthesis contains a gametophyte in some immature stage of development. Figure 19 illustrates a gametophyte that persisted to anthesis in the single-megaspore stage. Development is often arrested in the subsequent two-nucleate stage. Although these immature structures appear to be healthy, it is doubtful whether they would ever develop into mature embryo sacs before fertilization. Being examples of abnormal gametophyte development, the immature types are grouped with the collapsed type.

The other type of sterile ovule presents a rather enigmatic appearance and differs markedly from the collapsed and normal types. The cavity normally occupied by a fully developed embryo sac is filled, in this instance, by a mass of 20 to 40 relatively undifferentiated cells (fig. 17, 18). Being more vacuolate, these cells are conspicuous in contrast to the more densely cytoplasmic cells of the surrounding integument. This structure may vary in shape from narrow and elongate (fig. 17) to ovoid (fig. 18). Its volume is always considerably less than that of a normal embryo sac. Neither the vestiges of a nucellar epidermis nor any other structure separates this mass of cells from the inner layer of the integument. This very fact suggests

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that the abnormal structure is itself the nucellar apex, which has persisted to anthesis in the absence of any gametophyte. The same origin is indicated by the following developmental study. This type of sterile ovule is therefore known here as the *substitution* type.

Plants No. 16–4, 16–29, 16–38, and 16–56 produced only ovules of the substitution type (table 1). The inability of this type to develop into viable seed is attested by the fact that repeated application of viable pollen to stigmas of these plants failed to yield a single seed. Incompatibility reactions in the style were eliminated as a possible explanation of the sterility of these plants because pollen tubes were found in their ovarian cavities after pollination.

Ovules of the collapsed and substitution types are as large as normal ones and, except for their functionless contents, are as fully developed. Their structure is comparable in every respect save the inner layer of the integument and the contents of the embryo sac cavity. In both types the integumentary cells immediately surrounding the embryo sac cavity, particularly at the micropylar end, are much larger than other cells of the same integument and those of similar position in normal ovules; moreover, they are usually elongated radially, as if they had encroached centripetally upon the space that would have been occupied by a normal embryo sac (compare fig. 11, 12, 17, 18, and 20 with fig. 6).

Development of the normal (fertile) ovule.—The development of a particular structure is difficult to determine if a mixture of several developmental patterns appears in the same preparation. Here the study was greatly facilitated by the discovery of certain plants whose ovules are all or nearly all of a single type. Thus the development of normal-type ovules could be followed with confidence in fruitful diploids, the collapsed type in triploids, and the substitution type in such mutant diploids as No. 16–4 and 16–29.

Since the development of the fertile tomato ovule was found to correspond with the descriptions by Cooper (1931) and Smith (1935), the following brief description will suffice for comparisons with sterile ovules.

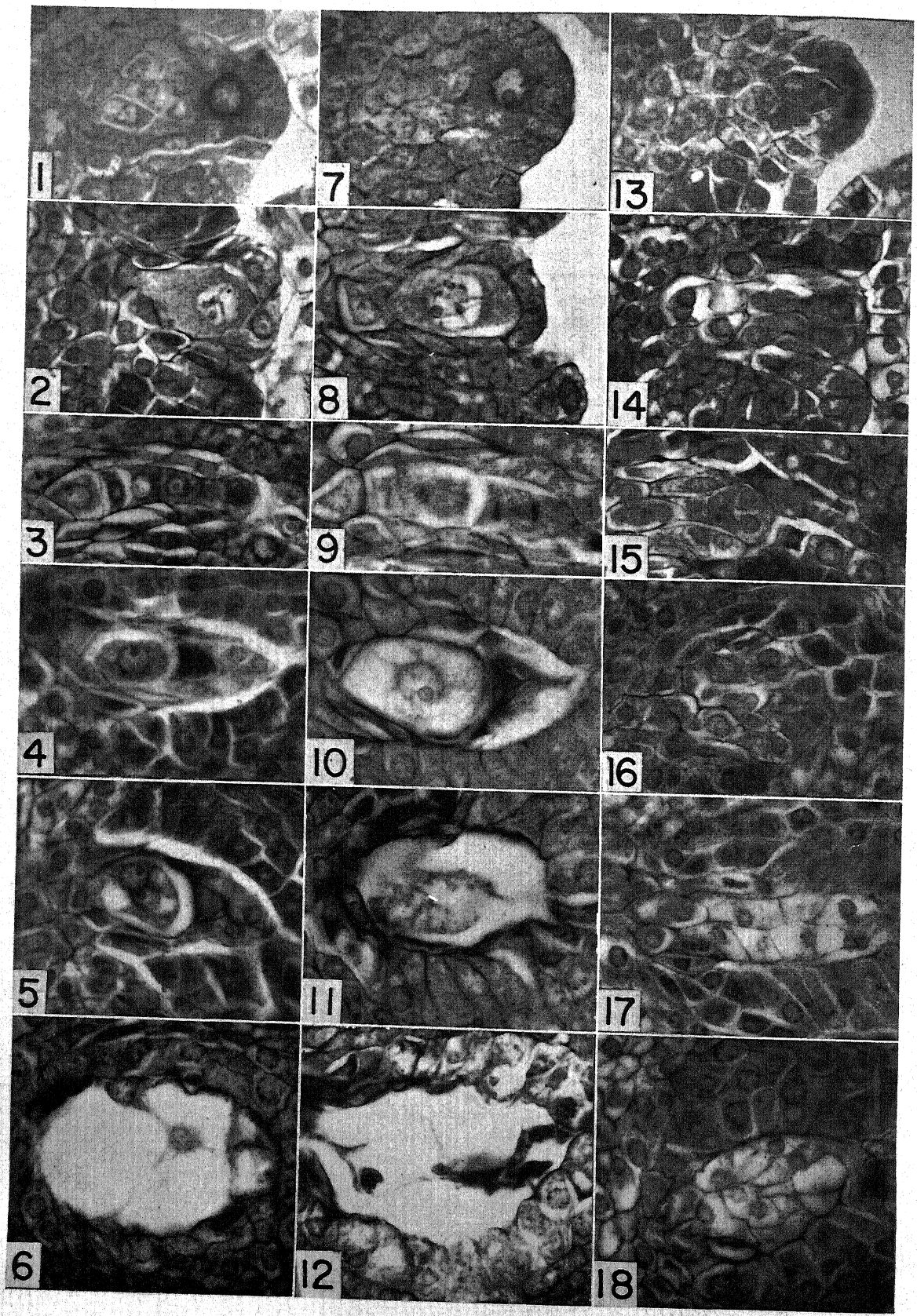
In the apex of the nucellus, a single hypodermal cell, well differentiated in early stages by its dark-staining cytoplasm (fig. 1), and later by its larger size and enlarging nucleus (fig. 2), develops directly as the megaspore mother cell, no division into parietal cell and sporogenous cell intervening. In a single ovule of a triploid tomato, two well-differentiated megaspore mother cells, whose radial walls were contiguous as if the two cells had been produced by anticlinal division of a single archesporial cell, constitute the only observed exception to this condition. The other cells of the nucellar apex, relatively few in number, remain as a single layer of cells investing the developing gametophyte. Subsequently the megaspore mother cell gives rise to an embryo sac according to the so-called normal-type development. In anthers of the same flower, pollen mother cells undergo meiosis, and microspores are released from tetrads before the first division of the megaspore mother cell. The latter elongates axially and by the two meiotic divisions produces a linear tetrad of megaspores, the one at the chalazal end being larger than the others (fig. 3). Soon after the second division is completed, the three megaspores at the micropylar end collapse to form small, dark-staining bodies, crescent-shaped in longitudinal section (fig. 4). By this stage the contents of the nucellar epidermal cells have largely disappeared, and this whole structure is compressed until it remains only as a thin layer of crushed cell walls surrounding the gametophyte. Subsequently the megaspore elongates axially, and three nuclear divisions follow in rapid order, giving rise to the eight-nucleate embryo sac. A two-nucleate embryo sac, the product of the first division, is illustrated in figure 5. The gametophyte rapidly increases in volume during these divisions. The eight nuclei orient themselves in their respective cells as previously described.

As the gametophyte develops, the single integument—an annular, encircling mass of ovular tissue—grows in the apical direction, completely surrounding the nucellar apex by the time the megaspore mother cell divides for the first time. Cell division continues until, at anthesis, the gametophyte is deeply imbedded within the eight to ten cell layers of the integument.

Development of the collapsed type.—Development of the collapsed type was studied in sectioned ovaries of triploids. Although ovules of triploids were not entirely of this type, 10 to 20 per cent being normal or substitution types, the course of development could be followed with a fair degree of certainty. Except the very small proportion belonging to the substitution type, all ovules of triploids develop normally up to the megaspore stage. Hence it can be assumed that the collapsed type develops normally at least to this stage. The appearance of ovules in subsequent development, as described in the following, leaves little doubt concerning the identity of collapsed-type ovules.

Photomicrographs of the developing collapsed-type ovules (fig. 7 to 12) are arranged parallel to those of the normal type (fig. 1 to 6); figures at the same horizontal level are of approximately the same developmental age, development proceeding from top to bottom. Figures 13 to 18 of the substitution-type series are arranged in a similar way.

A megaspore mother cell is differentiated in typical fashion (fig. 7, 8). The meiotic divisions proceed normally (fig. 9) except for occasional lagging chromosomes. Chromosomes are too small to permit accurate determination of pairing relations. Development nearly always continues beyond the four-megaspore stage, disintegration occurring during differentiation of the gametophyte. Breakdown may intervene at any time from the megaspore stage to the eight-nucleate embryo sac. This variation in


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stage of collapse is reflected in the wide range of size of the disintegrated structure at anthesis and in the corresponding variation in encroachment of the inner cells of the integument. Figure 20 illustrates a cross section of an ovule that collapsed in an early stage. Here the cavity is small, and surrounding cells of the integument have invaded centripetally to a great extent. Collapse at a later stage is suggested by the internal structure of the ovule in figure 12. Since bodies that resemble remnants of synergid cells appear at the micropylar end, this gametophyte may have reached the eight-nucleate stage before collapse. Corresponding to this late stage of development, the embryo sac cavity is nearly normal in size, and surrounding integumentary cells are less hypertrophied than in examples of earlier collapse. Presumably breakdown might start at any time up to the final stages of differentiation of a mature embryo sac.

The nucellar epidermis of collapsed-type ovules disintegrates as in corresponding stages of normal fertile ovules. Where an immature gametophyte persists to anthesis, the nucellar epidermis appears to remain in a correspondingly immature state (fig. 19). The remains of the nucellar epidermis can also be seen in figure 12.

In general features, collapse of the female gametophyte in tomatoes resembles that in female-sterile potato varieties (Arnason, 1943) and in female-sterile amphidiploids of *Nicotiana* (Greenleaf, 1941). Breakdown does not involve the meiotic process as in autotetraploid lettuce (Einset, 1944). Hypertrophy of the inner cells of the integument is a constant feature of the collapsed type, not only in all these instances, but also in other recorded examples.

Development of the substitution type.—From the earliest differentiation of reproductive structures until anthesis, the substitution type differs from the two types previously described. In ovules of the normal and collapsed types a megaspore mother cell has enlarged enough to be distinguished readily by the time that microspores first appear in anthers of the same flower (fig. 1, 2); in ovules of the same age in the substitution type a group of smaller cells, which cannot be distinguished by size or shape from other cells of the nucellus, usually appear in the place of the megaspore mother cell (fig. 13, 14). However, a "potential" megaspore mother cell—a cell somewhat larger than surrounding cells—is found in the nucellus of about ten per cent of the ovules of this age (fig. 21). Such potential megaspore mother cells always appear in a passive condition; in fact, division figures have not been found in any cells of this nucellar group after it has differentiated. In later stages evidence of degeneration of such potential megaspore mother cells (or any cells) of the nucellar apex was sought without success; they evidently develop in the same manner as the other cells of the nucellar apex. The developmental pattern followed by substitution-type ovules up to anthesis is very simple. The nucellar apex persists while it is being enveloped by the integument (fig. 15, 16). The cells gradually become more vacuolate, until at anthesis they appear hyaline in contrast to cells of the surrounding integument (fig. 17, 18). Instead of collapsing at an early stage, they continue to exhibit prominent nuclei and other features of living condition, at least until anthesis. This group of cells remains in constant contact with the surrounding integument; the two are never separated by a layer of disintegrating cells, as they are in normal ovules, where the disintegrating nucellar epidermis occurs between the integuments and the young gametophyte. No structure resembling a living or collapsed gametophyte was seen in many hundreds of these ovules. The abnormal structure occupying the central cavity of this type of sterile ovule therefore is the nucellar apex (that is, the nucellar epidermis at the apex and cells enclosed by this epidermis), which substitutes for the normally developed gametophyte.

The fundamental difference between the collapsed and substitution types thus lies in the time when abnormal development starts. If the breakdown occurs before meiosis, the substitution type develops; if the breakdown occurs in the post-meiotic period, the collapsed type is produced. Breakdown of the embryo sac, as of pollen in genetically conditioned male sterility, might presumably occur at any stage of development. Breakdown during the meiotic divisions might lead to the development of an intermediate type of sterile ovule; however, meiosis, once it is initiated, seems to proceed normally in all tomatoes studied here. The lack of interruptions at this stage thus seems to be responsible for the wide morphological gap between the two types of sterile ovules as they appear at anthesis.

Proportions of the three types of ovules in various plants.—Table 1 shows the frequencies of the three types of ovules—normal, collapsed, and sub-

Fig. 1–18. Photomicrographs of the central portion of longitudinal sections through tomato ovules. Micropylar end on right. ×600.—Fig. 1–6. Stages in development of normal-type ovules.—Fig. 1. Early differentiation of megaspore mother cell.—Fig. 2. Nucleus of megaspore mother cell in prophase of meiosis.—Fig. 3. Linear tetrad of megaspores.—Fig. 4. Chalazal megaspore enlarging; remaining megaspores crushed.—Fig. 5. Binucleate gametophyte.—Fig. 6. Embryo sac at anthesis, showing synergid cells and polar nuclei.—Fig. 7–12. Corresponding stages in development of collapsed-type ovules. Figures 10 and 11 represent somewhat later stages than figures 4 and 5 respectively. Ovules all from 3n plants.—Fig. 7. Early differentiation of megaspore mother cell.—Fig. 8. Nucleus of megaspore mother cell in prophase of meiosis.—Fig. 9. Late anaphase of second meiotic division.—Fig. 10. Enlarging megaspore.—Fig. 11. Collapse of gametophyte at an early stage.—Fig. 12. Collapse of older gametophyte.—Fig. 13–18. Corresponding stages in development of substitution-type ovules. Ovules from plants No. 16-4 and 16-29.—Figures 17 and 18 illustrate condition at anthesis.

TABLE 1. *Proportions of three types of ovules in fruitful and unfruitful plants.*

Plant No.	Variety	Nature of plant	Total ovules examined	Per cent ovules of: Normal type	Collapsed type	Substitution type
145–1	Early Santa Clara	Fruitful diploid	155	88	5	7
132–1	San Marzano	Fruitful diploid	196	97	2	1
16–46	Early Santa Clara	Triploid	151	8	87	5
16–40	San Marzano	Triploid	139	17	80	3
16–43	San Marzano	Tetraploid	179	43	53	4
117–1	Pearson	Tetraploid	238	24	74	3
16–9	Early Santa Clara	Apetalous mutant diploid	162	13	55	32
16–4	Early Santa Clara	Completely sterile diploid	249	0	0	100
16–29	Early Santa Clara	Completely sterile diploid	342	0	0	100
16–38	San Marzano	Completely sterile diploid	201	1	0	99
16–56	Early Santa Clara	Completely sterile diploid	273	0	0	100

stitution—in representative material. Three to six ovaries of each plant were scanned for ovule type. Although striking differences in the proportions of the three types of ovules exist between plants of different genotype and chromosome number, little variation in the proportions of each type was noted between ovaries of the same plant (except in No. 16–9, as mentioned subsequently). For reasons already mentioned (Rick, 1945), normal ovules probably cannot be identified so reliably as the sterile types. The collapsed and substitution ovules lack the structures essential for further development and must therefore be sterile. Pollination studies also indicate their inviability. Normal ovules in the preparations have a mature embryo sac and thus appear capable of developing into seeds pending fertilization, but it is doubtful whether one could identify all the derangements that might impair fertility, and whether all normal embryo sacs are functional. Judging from comparisons with seed fertility, identification of normal ovules in fixed preparations is reasonably accurate, though minor discrepancies probably exist.

According to table 1, both types of sterile ovules occur with a low frequency in fruitful diploid tomatoes. In triploids and tetraploids, the substitution type maintains the same low proportion, namely, one to five per cent, but the collapsed type includes the majority of ovules, thus accounting for nearly all the ovule sterility of these heteroploids.

Sterility of triploids is generally conceded to be caused by abortion of gametes that receive unbalanced chromosomal complements. Evidence in the literature for a similar explanation of sterility in autotetraploids is more contradictory. Although not discriminatory, the information in the present study is in keeping with this explanation for both polyploid types. According to this hypothesis, degeneration is expected in postmeiotic stages. This is precisely what is found: the great majority of their sterile ovules are of the collapsed type, in which disintegration occurs while the gametophyte is maturing. Furthermore, a breakdown conditioned by the genotype or chromosome number of the megaspore itself would be expected to appear at random in respect to the ovule's position on the placenta, whereas a maternal effect would be less likely to appear at random. The former condition is also fulfilled, since collapsed-type ovules, in contrast to the substitution type, appear on any part of the placenta and do not seem to be more numerous in some positions than in others.

In ovules of triploid *Datura Stramonium,* Satina and Blakeslee (1937) also find that meiosis pro-

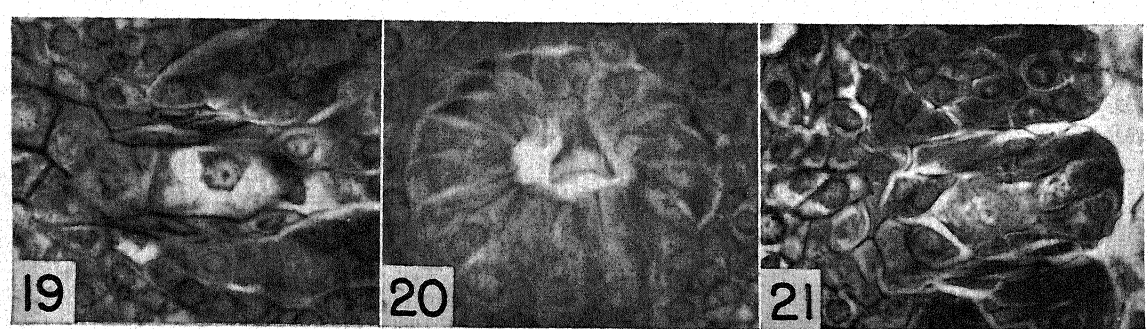


Fig. 19–21. Photomicrographs of the central portion of tomato ovules. ×600.—Fig. 19. Longitudinal section of a 3n ovule showing a single megaspore that has persisted to anthesis.—Fig. 20. Transverse section showing collapse in early development of gametophyte.—Fig. 21. Longitudinal section of substitution-type ovule of about the same age as that in figure 14, showing central cell larger than the rest.

ceeds normally except for elimination of lagging chromosomes. They likewise report examples of retarded development of the gametophyte. Although the *Datura* gametophytes rarely degenerate before the three mitotic divisions are completed, the majority have disintegrated or fail to be fertilized after pollination of the flower (Satina and Blakeslee, 1938). Comparisons of chromosome numbers in the female gametophyte and in seedlings of 3n × 2n crosses reveal a tremendous elimination of extrachromosomal types. Inviability of the female gametophyte probably accounts for part of this elimination.

Unbalanced chromosomal complements of the gametophyte itself obviously are not responsible for all examples of collapsed-type abortion. An adverse maternal environment can apparently lead to the same breakdown, since this type is also found in low frequencies in fruitful diploids and at higher frequencies in the mutant diploid No. 16–9.

The overgrowth of the inner integumentary cells of 3n and 4n ovules accompanying collapse of the gametophyte recalls the hyperplasia of maternal tissue in postfertilizational ovules of *Nicotiana rustica* × *N. glutinosa* described by Cooper and Brink (1940), of 2n × 4n, 4n × 2n *Lycopersicon pimpinellifolium,* and 2n *L. pimpinellifolium* × 2n *L. peruvianum* (Cooper and Brink, 1945), and of self-pollinated *Medicago sativa* (Brink and Cooper, 1940). These writers suggest that in a normal balance between endosperm and inner maternal tissue, the endosperm competes successfully, transferring nutrients from the ovule to the immature embryo. If the endosperm lacks its normal vigor because of inbreeding, incongruous interspecific mating, or improper chromosomal balance, the surrounding maternal tissues then become hyperplastic at the expense of the endosperm. Secondarily, the embryo starves and the ovule aborts. This somatoplastic sterility might be extended in a modified version to explain the failure of ovule development in prefertilizational stages in tomatoes. According to this explanation, aggression by the integument would be the primary cause of collapse. Inherent defectiveness of the gametophyte, however, for reasons given above, is the more likely primary cause. In respect to time of collapse, also, the gametophyte, rather than the surrounding soma, seems to play the dominant role; evidence of collapse normally precedes signs of abnormal activity in the integument. Furthermore, in the substitution type, hypertrophy of the inner layer of the integument is certainly a consequence, not a cause, of the aberrant nucellar behavior.

The substitution type appears in all investigated tomato plants; a low incidence of this type of sterile ovule is probably characteristic of all tomatoes, even those of normal fertility. When found at a low frequency, the substitution type appears mostly in the crowded parts of the ovary—chiefly in the inner placental folds, especially of the multilocular ovaries. Since these haunts of the substitution type seem less favorable, perhaps starvation, crowding, or some other unfavorable condition peculiar to these positions induces ovules to develop into the substitution type. This explanation is also suggested by the higher proportion of substitution-type ovules characteristic of fruitful diploids growing under certain conditions unfavorable to fruiting. Shortly after being transplanted to the field early in the growing season, tomato plants usually fail to produce fruit in the first inflorescence to flower after transplanting. The lowering of nutritional reserves as a result of transplanting probably accounts for this temporary unfruitfulness. In eight ovaries collected at this time from diploids that were fruitful later in the season, 15 per cent of 175 ovules were of the substitution type. These ovaries differed not only in their having a higher proportion, but also in a higher interovary variability of the proportion of substitution type. The proportion of these sterile ovules usually decreases to about five per cent as conditions become more favorable.

Of the five mutant diploids listed in table 1, one, No. 16–9, exhibits a mixture of three types of ovules, in which the sterile types predominate. A great reduction in development of the corolla and considerable deformation of other flower parts is associated with sterility in this mutant. These associated defects are apparently the spurious pleiotropic expression of a single gene. This explanation seems plausible in this instance because a fundamental disturbance caused by the gene in the early development of the flower conceivably might lead to unfavorable conditions for the subsequent formation of ovules as well as to abnormal gross morphology of the corolla and androecium. The interflower variation in distortion of flower parts is great and is matched by a correspondingly high fluctuation in the proportion of ovule types.

Ovules of the four remaining mutant diploids consist only of the substitution type. (Identification of a single ovule of normal type in plant No. 16–38 was uncertain.) These plants also exhibited high pollen abortion, and no seeds were produced when their gametes were used in pollination tests. Breakdown of pollen production resembled that of male-sterile mutants except in No. 16–38, in which, peculiarly, breakdown followed asynaptic meiosis in pollen mother cells. The complete sterility of pollen and ovules prevented any genetic study of these plants; however, it seems likely, for reasons already presented (Rick, 1945), that gene mutation or the absence of small chromosomal segments was responsible for the sterility.

Either unfavorable growing conditions or an unfavorable genotype of the sporophyte can therefore stimulate ovules to develop into the substitution type. Although both types of ovule breakdown can be genetically determined, they differ in the mode of genetic control. Whereas the chromosomal condition of the gametophyte itself is apparently responsible in most instances for abortion of the col-

lapsed type, the genotype of the maternal tissue determines the substitution type.

SUMMARY

Ovule abortion in genetically unfruitful tomato plants was found to consist of two types.

In one, the *collapsed* type, the ovule develops normally until four megaspores are produced. Thereafter, the gametophyte may degenerate in various stages until differentiation of a mature embryo sac. Collapse of the gametophyte is accompanied by hypertrophy of the inner layer of integumentary cells. Triploids and tetraploids are characterized by ovule sterility of the collapsed type. In most instances development of this type is probably conditioned by adverse chromosomal unbalance of the gametophyte itself. The same type of breakdown can also be induced apparently by an adverse maternal environment.

In the other, the *substitution* type, a megaspore mother cell never differentiates, nor is there any semblance of normal development of a gametophyte. Instead, the nucellar apex remains as a group of living, undifferentiated cells, which persists in the ovule until anthesis. Although the inner integumentary cells also hypertrophy, the outer ovule tissues develop normally. At least a low frequency of substitution-type ovules is characteristic of all plants examined, fruitful as well as unfruitful. Certain sterile mutants produce ovules solely of this type. Unfavorable conditions in certain positions in the ovary of all tomatoes and in all positions in certain mutants apparently lead to this type of development.

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NEW AND NOTEWORTHY ASIATIC SPECIES OF LESPEDEZA [1]

P. L. Ricker

FURTHER STUDIES on the species of Asiatic *Lespedeza* and the opportunity to examine 400 sheets of recent acquisitions by the Arnold Arboretum have given a much better idea of the distribution, relationships and variations in a number of species, and have enabled the reduction of a few species that had previously been considered new.

Scarcity of material of most of the species and the impossibility of field study of the remaining Asiatic material to determine the limits of variation make it desirable to publish notes at present available for the benefit of any who may, after present disturbed conditions, have an opportunity of making a field study of this interesting genus in Asia. To anyone having such an opportunity it is urged that scale drawings be made of the calyx with particular attention to all of the calyx teeth, and that basal sterile leafy branches be collected with flowering leafy branches and so numbered and labeled that it may be readily possible for anyone to visualize beyond question the limits of leaf variation in a single plant. For example, H. H. Chung 2302 and 9062 with oblanceolate leaflets were considered a new species until his number 6645 was later received for study and was found to have the oblanceolate terminal leaflet and basal broadly ovate, much larger leaflets on the same branch and identical with those of *L. davidii* Franch., though such variation does not show on other sheets, or in material grown from seed in this country. Without accompanying notes it has been impossible to give the approximate height of some of the species.

Photographs of type sheets and a natural size photograph of the flowers, leaves and pods of the new species here described, have been deposited in the herbaria of the New York Botanical Garden, Bailey Hortorium, Arnold Arboretum, Gray Her-

[1] Received for publication December 14, 1945.

barium and Missouri Botanical Garden (unless the type sheets have been designated in these herbaria) and are available in exchange for type photographs from other large herbaria.

LESPEDEZA (EULESPEDEZA) **mucronata** Ricker, sp. nov.

Shrubby, about 6 dm. tall; stems erect, tomentose above, sparingly so below. Stipules linear-lanceolate, about 3 mm. long; leaflets obovate to obcordate, puberulent above, pubescent below, up to 9 × 13 mm. Flowers few, axillary; calyx tomentose, about 4 mm. long, tube 1 mm. long, teeth hirsute, aristate, about 3 mm. long; corolla about 6–7 mm. long, yellow; pod about 2 × 3 mm. long, elliptic, acute; mucro, 2–4 mm. long.

Suffrutex, caule erecto, ca. 6 dm. alto, tomentoso. Stipulae lineari-lanceolatae, ca. 3 mm. longae; foliolis obovatatis vel obcordatatis, usque ad 9 × 13 mm. Flores axillares; calyce tomentoso, ca. 4 mm. longo, laciniis aristatis ca. 3 mm. longis; corolla ca. 6–7 mm. longa, flava.

Type, H. H. Chung No. 5384, Amoy, Nanputo, Province of Fukien, China, Nov. 21, 1926, in herbarium of the Arnold Arboretum. Not resembling any other species of Eulespedeza. The collector gives the flowers as yellow, but they have dried much darker-colored than yellow flowers usually do, suggesting that part of the flower at least may have been purple or purplish-spotted as are several of the yellow-flowered Asiatic species of Eulespedeza. Most nearly related to *L. elegans* Cambess., but much less tomentose, leaflets smaller and apex retuse instead of obtuse.

LESPEDEZA (MACROLESPEDEZA) **anthobotrya** Ricker, sp. nov.

Erect shrub, branches slender, spreading, angled, pubescent; stipules filiform, 3 mm. long. Petioles up to 4 cm. long, about equaling the leaves, petiolules of lateral leaflets up to 2.5 mm., long hispid, the terminal leaflets up to 10 mm. long; upper leaflets narrowly elliptic with acute base, up to 1.2 × 2.7 cm.; lower leaflets obovate and up to 1.5 × 3 cm., glabrous above, puberulent below. Flowers 4–8 in nearly sessile, mostly terminal, clusters; calyx hispid, 4–5 mm. long, teeth acuminate, equaling the tube, the dorsal tooth bifid; corolla about 9 mm. long, reddish-purple. Fruits unknown.

Frutex erectus, ramis angulatis diffusis. Foliorum superiorum foliola anguste elliptica, 1.2 × 2.7 cm., foliorum inferiorum foliola obovata, ca. 1.5 × 3 cm., supra glabra, subter puberulenta. Flores 4–8 subsessiles; calyce 4–5 mm. longo, laciniis acuminatis, tubum aequantibus; corolla ca. 9 mm. longa, rubropurpurea.

Type, K. Shiota, No. 7854, Mino Province, Hondo, Japan, Aug. 29, 1934, in herbarium of the Arnold Arboretum. *Lespedeza anthobotrya*, which suggests *L. satsumensis* Nakai, has much thinner and somewhat narrower leaflets and stems not tomentose. The broader obovate leaflets at the base of one specimen of *L. anthobotrya* suggests also a close relationship to *L. crytobotrya* Miq. in the nearly sessile flower clusters, but the terminal leaflet is not quite as broad and leaflets available are not at all retuse at the apex as in *L. cyrtobotrya*.

LESPEDEZA (MACROLESPEDEZA) **albiflora** Ricker, sp. nov.

Woody shrub, erect, 3 ft. tall, stems appressed-pubescent; leaflets obovate, up to 1.9 × 3.0 cm., glabrous to puberulent above, sparingly puberulent below; racemes up to 10 cm. long, arcuate to pendulous; corolla 9–10 mm. long, white; calyx 3–4 mm. long, teeth equaling the tube, acute, the upper with bifid teeth 1 mm. long.

Suffrutux erectus, 1 dm. altus. Foliola obovata, ca. 1.9 × 3.0 cm., supra glabra vel puberulenta. Racemi usque ad 10 cm. longi, arcuati usque penduli; corolla alba, 9–10 mm. longa; calyce 3–4 mm. longo; laciniis tubum aequantibus.

Type, W. T. Tsang, No. 25896, paratype No. 25664, Kwantung, China, Sept.-Oct., 1935, in herbarium of the Arnold Arboretum.

In the more than 1000 sheets of Asiatic Lespedezas examined from the larger American herbaria, no other specimens have been found at all approaching this species. It may be identical with *Desmodium racemosum* var. *albiflorum* Sieb.[2] *L. albiflora* has the leaflets of *L. cyrtobotrya* Miq., and the flowers and pendulous branches of *L. thunbergii*, which has elliptic-lanceolate leaflets, well figured by Oudemans[2] as *Desmodium racemosum*. These characters suggest that *L. albiflora* may be a possible hybrid between *L. thunbergii* and *L. cyrtobotrya* Miq., or the white-flowered *L. japonica* may be involved. We have been unable to find any material of a white-flowered form of *L. thunbergii* (DC.) Nakai with the typical narrowly elliptic-lanceolate leaflets of that species and find no evidence that a white-flowered *Lespedeza* with obovate leaflets and much shorter calyx teeth should be considered a variety of it. Previous original descriptions of white-flowered Lespedezas give no indications as to their leaf shape, and Nakai[3] does not mention any white-flowered forms in Japan or Korea.

Schindler[4] has thrown together a veritable hodgepodge of unrelated species under the name *L. formosa* (Vogel) Koehne. Koehne's distributed specimens are a perfect match for the typical *L. thunbergii* and Oudemans' figure, and the calyx-teeth are quite distinct from those of *L. formosa*.

The original description[5] of *Desmodium formosum* Vogel calls for "foliolis 3 subovalibus parum emarginatis mucronatis." The leaflets of the true *L. thunbergii* to which Schindler refers it are never "emarginatis mucronatis" so it can not be a synonym of that species. The type locality is Macao on an island near the mouth of the Si-kiang River in China. The type of *L. thunbergii*, without specific locality, is from Japan, where it has been cultivated for a

[2] Oudem. Neerl. Plant 2: pl. 2. 1866.
[3] Nakai, T. Lespedezas of Japan and Korea, 1927.
[4] Bot. Jahrb. 49: 580–581. 1913.
[5] Nov. Act. Acad. Caes. Leop. Car. Nat. Cur. 19: 29. 1843.

long time. All specimens examined that seem to be of probable wild origin have been from areas of Sze-chwan, Anhwei and Kiang-si just south of the Yang-tze-kiank River, and one dubious wild locality from extreme southern Japan. It is quite possible that much of the Japanese material may have come originally from China.

In the same mix-up is involved *L. viatorum* Champ[6], type from Hong-kong, about 50 miles from Macao, with more or less obovate and emarginate leaflets. It is close to *L. formosa* and may be a synonym, but until the type or detailed type photographs can be critically examined, it is impossible to say.

LESPEDEZA (MACROLESPEDEZA) JAPONICA **ovata** Ricker, var. nov.

A compact shrub up to 2 m. tall, with arcuate branches. Upper leaflets small and obovate to oblanceolate, lower leaflets ovate to lanceolate, up to 2.5 × 5 cm., glabrous above, densely cinerous and prominently veined below. Racemes mostly short and densely flowered, not much exceeding the leaves; bracteoles broadly acute, less than half the length of the calyx-tube; flowers similar to those of the species but more numerous, compact and white. The plants grown from seed often have somewhat fasciated branches and the shrubs are then more compact than normal.

Frutex densus, usque ad 2 m. altus. Foliola usque ad 2.5 × 5 cm., obovata vel ovato-lanceolata, dense cinerea. Corolla alba, bracteolis late-acutis, calycis dimidium tubum haud aequantes.

Type, S. P. I. 56524 grown from seed received from Dr. Takeo Kusano, Kagoshima, Japan, in 1923, collected by P. L. Ricker at Arlington Farm, Va., Sept., 1933, in herbarium of the U. S. National Arboretum.

Readily distinguished from the species, which is also white-flowered, by its more compact leaves and flowers. The bracteoles in *L. japonica* are linear-lanceolate and about equal the tube instead of being broadly acute as in *L. japonica* var. *ovata*.

Nakai[7] erroneously describes Bailey's white type, *L. japonica*, as *L. japonica* var. *albiflora* and the purple-flowered variety as *L. japonica* var. *intermedia*.

LESPEDEZA (MACROLESPEDEZA) **metcalfi**[8] Ricker, sp. nov.

Shrubby, stem 2 ft. high, much branched, appressed-pilose. Stipules linear-lanceolate, about 4 mm. long; petioles about 2 cm. long; leaflets elliptic to oblanceolate, up to 2 × 5.5 cm., densely pubescent below, puberulent to glabrous above, apex mucronate. Peduncle slender, 1.5–2 cm. long, racemes axillary, few-flowered, 2–3 cm. long; calyx 7–8 mm. long, tube 2 mm. long, teeth linear-lanceolate, appressed-pubescent; corolla 10–12 mm. long, reddish-purple, wings and keel 8–10 mm. long. Pods unknown.

Suffrutex, 6 dm. altus, multiramosus, adpresse pilosus. Stipulae lineari-lanceolatae ca. 4 mm. longae; petiolo ca. 2 cm. longo; foliolis ellipticis usque oblanceolatis, usque ad 2 × 5.5 cm. Pedunculus tenuis 1.5–2 cm. longus. Racemi axillares, sparsiflori, 2–3 cm. longi; calyx 7–8 mm. longus, tubo 2 mm. longo, laciniis lineari-lanceolatis pubescentibus; corolla 10–12 mm. longa, rubro-purpurea, alis et carina 8–10 mm. longis. Legumen ignotum.

Type in herbarium of the Arnold Arboretum, C. S. Fan and Y. Y. Li No. 215, Providence of Anhwei, China, June 24, 1936.

Most closely resembling *Lespedeza dunni* Schindl., but differs in its much larger leaflets and flowers, apex of leaflets not retuse, fewer-flowered and shorter raceme. The leaves somewhat resemble those of *L. stottsae* Bailey.

LESPEDEZA (MACROLESPEDEZA) **paradoxa** Ricker, sp. nov.

Shrubby, stems erect, slender, flexuous, 3–6 dm. tall, reddish, glabrous. Stipules linear-lanceolate, about 3–6 mm. long; petioles about 1 cm. long, puberulent; leaflets ovate to lanceolate, mucronate, glabrous above, puberulent below, up to 1.6 × 4.7 cm. Flowers few, 2 cm. long, nearly sessile, or in slender racemes clustered in the axils; calyx 3.5–4.5 mm. long, strigose, teeth linear-subulate, 3–4 times longer than the tube; corolla 7–8 mm. long, purplish; pod elliptic to oblanceolate, acute at both ends, appressed-pubescent, about 4 × 13 mm.

Suffrutex, caule erecto 3–6 dm. alto. Foliola ovata vel lanceolata, usque ad 1.6 × 4.7 cm. Flores pauci, subsessiles, 2 cm. longi; calyce 3.5–4.5 mm. longo, strigoso; laciniis lineari-subulatis, tubo 3–4-plo longioribus; corolla 7–8 mm. longa, purpurea. Legumen ellipticum ad oblanceolatum, acutum, ca. 4 × 13 mm.

Type No. 1174 S. C. Sun, Chiuwashan, Anhwei, China, Aug. 17, 1933, in herbarium of the Arnold Arboretum and duplicate at the New York Botanical Garden. Not closely related to any described species but in pod character approaching that of *L. thunbergii*, the pods of both species resembling those of *Campylotropis* rather than those of any other species of the Macrolespedeza section. The flowers of this species are distinctly two at an axis except where one is broken off, and the keel is not geniculate.

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[6] Hook. Kew Journ. 4:47. 1852.

[7] Nakai, T. Lespedezas of Japan and Korea: 22–25. 1927.

[8] Named for Dr. F. P. Metcalf, formerly Professor of Botany at Lingnan University, Canton, China, and author of Flora of Fukien.

PLANT NUTRITION IN RELATION TO DISEASE DEVELOPMENT. III. FUSARIUM WILT OF TOMATO [1]

J. C. Walker and R. E. Foster[2]

EARLIER WORK on the relation of host nutrition to the development of vascular *Fusarium* diseases was reviewed in the first paper of this series (Walker and Hooker, 1945a). In that paper, it was shown that in a susceptible strain of cabbage there was a progressive decline in the rate of development of cabbage yellows (*Fusarium oxysporum* f. *conglutinans* (Wr.) S. & H.) with an increase in the salt concentration of a balanced nutrient solution. The disease progressed more rapidly at 25°C. than at 19°, but the same type of response to host nutrition occurred at both temperatures. In an intermediate resistant strain of the host, disease development was very slow at 19°, and no effect of host nutrition upon disease development could be shown. At 25°, which temperature was near the optimum for yellows, the response of the intermediate resistant host followed closely that of the susceptible strain at 19°. In the latter, omission of potassium from the culture solution resulted in an increase in disease development at both temperatures. Omission of nitrogen or phosphorus caused a decrease in disease development in the susceptible strain at 19° and at 25° and in the moderately resistant strain at 25°. A highly resistant strain of the host showed no disease development under any set of temperature or nutrient conditions.

Fisher (1935) reported that *Fusarium* wilt of tomato (*F. oxysporum* f. *lycopersici* (Sacc.) S. & H.) increased in severity in Bonny Best and Marglobe tomatoes grown with an excess of nitrogen, boron, or sulfur, while it decreased with a deficiency of these elements and with excesses or deficiencies of either magnesium or phosphorus. With either an excess or a deficiency of potassium, disease development decreased in Bonny Best, a very susceptible variety, but it increased in Marglobe, a resistant variety. Cook (1937), using older plants or cuttings, showed that, in general, high frequency of infection and symptom expression of *Fusarium* wilt of tomato were correlated with increase of nitrate in the nutrient.

The present investigation was concerned with a study of the relation of nutrient salt concentration and nutrient ion balance to tomato *Fusarium* wilt, conducted along the lines already reported with cabbage yellows (Walker and Hooker, 1945a) and cabbage clubroot (Walker and Hooker, 1945b).

METHODS AND MATERIALS.—Three types of host plant material were selected on the basis of inherent resistance or susceptibility to the disease. Bonny Best (*Lycopersicon esculentum* Mill.) was used as a representative of a very susceptible variety. Marglobe and Master Marglobe (*L. esculentum* Mill.) were used as representatives of commercial varieties that are intermediate in resistance. These show considerable resistance to the disease under most field conditions, but under conditions optimum for disease development they may appear to be quite susceptible. A strain of Red Currant tomato (*L. pimpinellifolium* Mill.) was used that was highly resistant to the disease. This strain was homozygous for the resistance gene described by Bohn and Tucker (1940).

Seeds were planted in shallow, enameled pans of washed, sterile, fine, white silica sand. Seedlings were watered on alternate days with distilled water or the nutrient solution described below as 0.1H. Plants of uniform size were transplanted into clean silica sand in drip-system pots as described by Walker and Hooker (1945a). Ten young plants were grown in each pot, and two or more replicated pots were used for each treatment. While the plants used were the same age in any given experiment, they were not the same age in all experiments, and one nutrient concentration experiment was designed to reveal any differences in results that might arise due to this condition.

The basal nutrient solution was identical with that used by Walker and Hooker (1945a) and designated by them as 1H. This balanced nutrient was reduced in concentration of elements to one-tenth (0.1H), and one-half (0.5H), and increased by two times (2H), and by three times (3H). In the experiments with unbalanced nutrient solutions, the basal 1H solution was used except that either N^+ or K^+ was increased to the amount found in the 3H solution (referred to as either "high N" or "high K") or reduced to that of the 0.1H level ("low N" or "low K"). Thus in the high N solution, 30 times as much nitrogen was present as in the low N solution. All other necessary ions were kept at the 1H level. None of the varied elements were ever lacking completely. In the experiments with unbalanced nutrients, all solutions were regulated to the same osmotic concentration by the addition of proper amounts of NaCl. In no solution did the total Na^+ or Cl^- concentration approach the amount readily tolerated by tomato plants. In all experiments the nutrient solutions were adjusted to pH 6.5 to 7.0. All were prepared with the same amount of minor element stock solution to eliminate the possibility of minor element deficiencies or excesses. Fresh solution dripped into each pot constantly at the rate of about 1200 to 1300 cc. per day and drained off after reaching a certain level in the pot by an automatic intermittent siphon.

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[2] The writers express sincere appreciation to Eugene H. Herrling for the preparation of illustrations used in this paper.

TABLE 1. *Relation of nutrient concentration to progression of Fusarium wilt in susceptible and intermediate resistant varieties of tomato.*

Nutrient concentration	Disease index at period indicated (days after inoculation)									
	Susceptible (Bonny Best)					Resistant (Marglobe)				
	10	13	17	24	38	10	13	17	24	38
0.1H	58	81	90	95	95	41	65	76	86	87
1H	23	58	77	87	89	22	47	49	63	63
2H	10	46	61	80	84	14	36	40	55	56
3H	21	50	63	82	88	14	40	48	67	67

After plants had grown for about two weeks, they were dug up and the roots were washed and dipped into aliquots of a heavy suspension of mycelium and spores of the fungus. The plants were then replanted into the same pots and furnished with the same nutrient solutions.

A virulent strain of *F. oxysporum* f. *lycopersici* secured from F. L. Wellman under number R 5–6 (Wellman and Blaisdell, 1940) was used as inoculum. The fungus was grown in liquid culture, the fungus mats were filtered off, washed, and macerated in distilled water in a Waring Blendor. The suspension so obtained was adjusted to approximately 20,000 viable conidia per mm^3 plus myriads of mycelial fragments. It was used immediately after preparation to prevent injury to the plants by nascent toxin. The superabundance of viable spores and mycelial fragments and their close proximity to all roots precluded the appearance of any differences due to the inoculation technique and resulted in uniform infection and rapid development of wilt with symptom expression typical of optimum environmental conditions.

In one nutrient concentration experiment, there was used also a very mildly pathogenic strain of the organism received from F. L. Wellman as A 15-8. This strain was grown and applied in the same manner as outlined above.

Greenhouse air temperature was maintained at 28°C., the optimum for disease development as reported by Clayton (1923). Shortly after the first appearance of wilt symptoms and at regular intervals thereafter, the plants were examined and each was assigned to one of the following disease classes: healthy, slightly wilted, moderately wilted, severely wilted and dead. For each host type under each nutrient condition a disease index was calculated as follows: the number of plants in the first class was multiplied by 0, that in the second class by 1, in the third class by 2, in the fourth class by 3, in the fifth class by 4. The sum of the products multiplied by 100 was divided by 4 × the total number of plants to secure the index. Thus the indices secured might range from 0, indicating all plants healthy, to 100, indicating all dead.

EXPERIMENTAL RESULTS.—*Concentration of balanced nutrient.*—From nine separate experiments conducted at different seasons of the year and all yielding similar results, five will be presented representing several minor variations.

Experiment 1. Bonny Best and Marglobe tomatoes, transplanted 17 days after sowing, were grown for 15 days in solutions of various concentrations and then inoculated. The solutions used were 0.1H, 1H, 2H, and 3H. Ten days after inoculation with the virulent strain of the organism, the first index

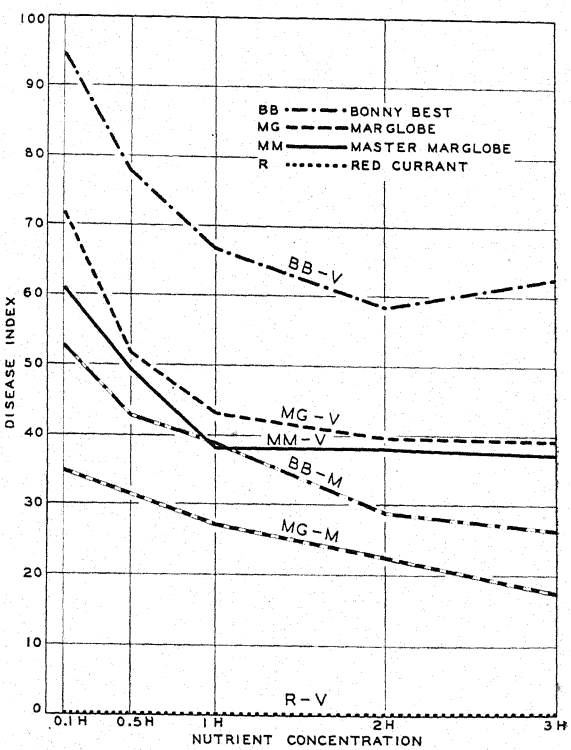


Fig. 1. Relation of wilt development in 4 host types to various concentrations of balanced nutrient 39 days after inoculation with virulent (v) and mild (m) strains of the pathogen.

readings were taken. These indices, as well as those from subsequent readings, are presented in table 1. In both the susceptible and intermediate resistant varieties, disease development was most severe in plants grown in the 0.1H solution. An increase in nutrient concentration was accompanied by a decrease in the rate of wilt development with the greatest differences occurring between the 0.1H and 1H solutions. There appeared to be a slight increase in severity of disease in the 3H nutrient over the 2H, although such differences were small. This experi-

TABLE 2. *Relation of nutrient concentration to progression of Fusarium wilt in a susceptible variety transplanted at various ages, but receiving variations in nutrient concentration for the same period of time.*

Nutrient concentration	Disease index at period indicated (days after inoculation) in Bonny Best plants of the various ages at time of transplanting																	
	12-day-old plants						18-day-old plants						26-day-old plants					
	8	12	16	20	24	28	8	12	16	20	24	28	8	12	16	20	24	28
0.1H	9	25	44	55	71	95	9	33	60	71	82	98	9	23	56	67	81	99
0.5H	5	18	38	51	69	90	5	28	51	67	81	96	8	18	45	63	77	91
1H	3	16	35	49	64	85	4	18	47	60	75	91	5	16	40	57	73	88
2H	0	10	31	38	50	78	1	6	18	36	66	81	2	12	30	48	57	74
3H	0	8	29	36	51	80	2	7	18	37	63	80	2	13	33	48	56	73

ment was conducted in August, September, and October.

Experiment 2. This experiment included Bonny Best, Marglobe, Master Marglobe, and Red Currant all grown in five different balanced-nutrient concentrations. Plants of all varieties were inoculated with the virulent strain of the fungus. At the same time, Bonny Best and Marglobe plants were inoculated with the mild strain. Disease indices taken on the thirty-ninth day after inoculation are presented for the various treatments in graphic form in figure 1. The period of this experiment extended through January, February, and March.

In plants inoculated with the virulent strain, wilt developed most severely in Bonny Best and to a lesser degree in Marglobe and Master Marglobe. The mild strain of the organism incited a milder form of wilt in each variety, although the relative susceptibility of Bonny Best compared to Marglobe was still apparent. Bonny Best infected with the mild strain developed less disease than Master Marglobe inoculated with the virulent strain which showed that differences in pathogenicity of the parasite had more effect upon disease reaction than did differences in host susceptibility. In susceptible or intermediate resistant hosts, wilt development had the same relationship to balanced nutrient concentration as was shown in Experiment 1 regardless of the pathogenicity of the organism used. A reduction in the severity of disease was occasioned by an increase in nutrient concentration with the greatest differences appearing between the 0.1H, 0.5H, and 1H levels and less reduction of disease in the 2H and 3H solutions. Bonny Best infected with the virulent strain showed a slight increase in disease development at the 3H level. Red Currant showed no disease development at any of the nutrient concentrations.

Experiment 3. Bonny Best and Marglobe plants were sown at three different times and transplanted simultaneously into drip-system pots. All, however, received the five levels of nutrient for 20 days before inoculation. Disease indices were taken on the same days and are presented for Bonny Best plants of different ages in table 2. Reactions were not consistently or significantly different between tomatoes of different ages. Plants of both varieties and of all three ages showed the same relative effect of nutrient concentration upon disease development, and this relationship was similar to that demonstrated in other experiments. Experiment 3 was conducted in October, November, and December.

Experiment 4. In order to simulate methods used by other workers in similar studies, a variation in the inoculation technique was made by applying inoculum (obtained as before) around the roots of undisturbed plants. One hundred ml. of spore and mycelium suspension were applied to each pot on three successive days. Bonny Best plants of three different ages when transplanted were inoculated after receiving the five different concentrations of balanced nutrient solution for 23 days. The disease appeared more slowly and less uniformly than in plants which were dip-inoculated. Disease indices were obtained 24 days after the first inoculation by cutting the plants and rating the extent of vascular discoloration. The height of discoloration was considered to be a more accurate indication of host-parasite interaction than the number of vascular bundles colored, the latter being regarded as a function of the number of points of infection. In rating the extent of disease in these plants, an effort was made to give indices approximating those assigned in other experiments. The data from this experiment conducted during September, October, and November are presented in table 3. While the disease development was not as rapid with this method of inoculation and the rating of disease probably was not as valid as in previous experiments, the relationship of nutrient concentration to disease development was found to be the same in this experiment as in previous ones. The progression of disease was most extensive in plants grown in the lowest concentration of nutrient, and an increase in nutrient level was accompanied by decreased disease development.

Experiment 5. In this experiment, diffusion pressure deficit (D. P. D.) of cells of the host plant was studied in relation to growth of the host, growth of the fungus and rate of disease development at various nutrient concentrations. Bonny Best plants growing in 0.1H, 0.5H, 1H, 2H and 3H solutions were dip-inoculated with the virulent strain of the organism, and disease indices were determined as in previous experiments. Control plants were grown and handled in the same way, except that at the time of inoculation they were dipped into distilled water

instead of into the fungus suspension. Toward the end of the experiment these healthy plants were removed from the pots, and the green weights recorded as a measure of total growth. At the same time the D. P. D. of these control plants was determined by a modification of the method outlined by Molz (1926a, 1926b). To secure a measure of fungus growth in the various nutrient solutions, 2 per cent dextrose was added to a quantity of each. The solutions were each divided into aliquots in Erlenmeyer flasks, autoclaved, cooled, seeded with a spore suspension of the fungus and incubated on an agitator for ten days at 28°C. The average dry weights of the fungus mats from the various nutrient solutions were taken as the measure of fungus growth. This experiment covered the months of December, January, and February.

TABLE 3. *Relation of nutrient concentration to progression of Fusarium wilt in Bonny Best of three different ages inoculated by applying the organism around the roots of undisturbed plants; disease indices obtained on the 24th day after inoculation by rating extent of vascular discoloration.*

Nutrient concentration	Disease index in Bonny Best plants of the ages indicated at time of transplanting		
	12 days	18 days	26 days
0.1H	14.8	17.0	15.8
0.5H	13.8	14.5	12.1
1H	10.5	12.4	9.7
2H	4.3	8.1	8.0
3H	4.0	7.7	6.1

The results are presented graphically in figure 2. The D. P. D. values of healthy plants increased with an increase in nutrient concentration up to 2H. Increase of D. P. D. was thus inversely proportional to rate of disease development. Total growth of healthy plants was affected by nutrient concentration but it was not correlated with D. P. D. or disease development. Fungus growth increased with increased nutrient concentration up to 1H, decreasing slightly at 2H and 3H. There was, therefore, no correlation between fungus growth and disease development.

BALANCED AND UNBALANCED NUTRIENT.—Bonny Best plants, grown, transplanted, and inoculated as before, received five different nutrient solutions varying in nitrogen or potassium content, normal (1H), high N, low N, high K, and low K. Disease indices were obtained as before and are presented in figure 3 for one experiment conducted in October, November and December. Experiments run in August, September, and October and in January, February, and March gave similar results. The development of wilt was greatest in plants receiving a nutrient solution low in potassium. The nutrient high in nitrogen also favored progression of disease over that obtained in plants given the normal solution. Solutions high in potassium or low in nitrogen were less favorable to wilt, plants in the low nitrogen nutrient solution showing the least development of the disease.

Growth of uninoculated host plants was influenced by the particular nutrient in which they were grown, but growth was not correlated consistently with relative susceptibility of plants grown in the various solutions. Average green weights of control plants revealed that best host growth was obtained in the high K solution. Order of decreased plant growth in the various solutions was as follows: high K, high N, normal, low K, low N, while increased susceptibility was brought about by the solutions in the following order: low N, high K, normal, high N, low K.

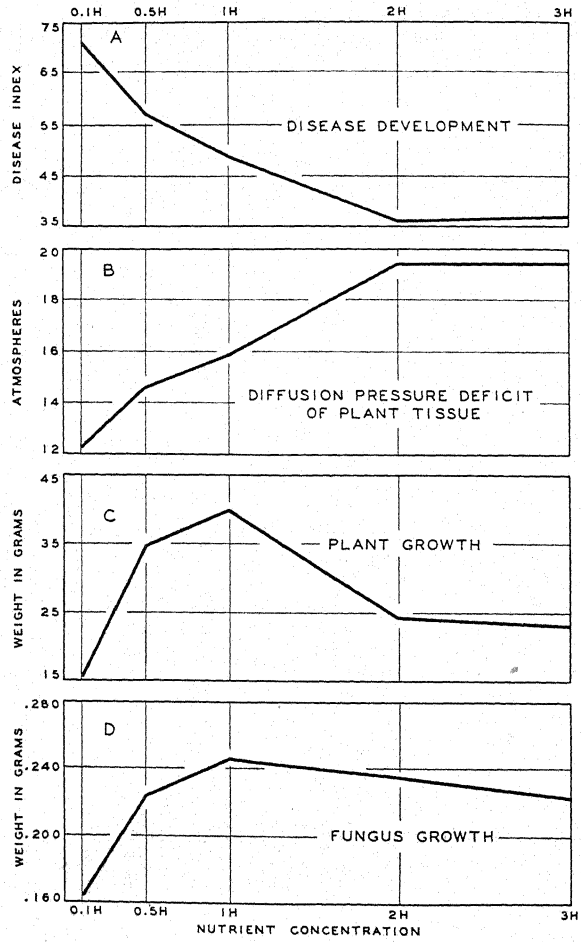


Fig. 2. Disease development in plants 19 days after inoculation (A), diffusion pressure deficit of healthy plants (B), growth of healthy plants (C), and fungus growth (D) in relation to nutrient concentration.

DISCUSSION.—*Fusarium* wilt of tomato is a hypoplastic disease similar in most respects to vascular fusarioses of various other plants. It differs from some in this group in that little or no cortical necrosis of the root system occurs under any set of environmental conditions that have been provided. Growth of the pathogen, growth of the young host

plants and wilt development have about the same optimum temperature.

As the salts in the complete balanced nutrient solution were increased from very low concentration (0.1H) to one ten times greater (1H), there was always a decided lag in the rate of disease development at the higher concentration. This lag increased somewhat when the concentration was increased to 20 times the lowest concentration (2H), while at 30 times (3H) the lag decreased slightly, but not significantly, when compared with 2H. When the salt concentration was kept at 1H and a uniform osmotic concentration maintained but the potassium and nitrogen levels varied, a definite influence on wilt development occurred. Low potassium or high nitrogen increased the rate of development while high potassium or low nitrogen retarded wilt.

In general, tomato wilt responded in these experiments to variation of nutrient concentration and balance in a manner similar to that of cabbage yellows, as described earlier (Walker and Hooker, 1945a). It appears that the vascular fusarioses follow a definite pattern in their response to nutrition

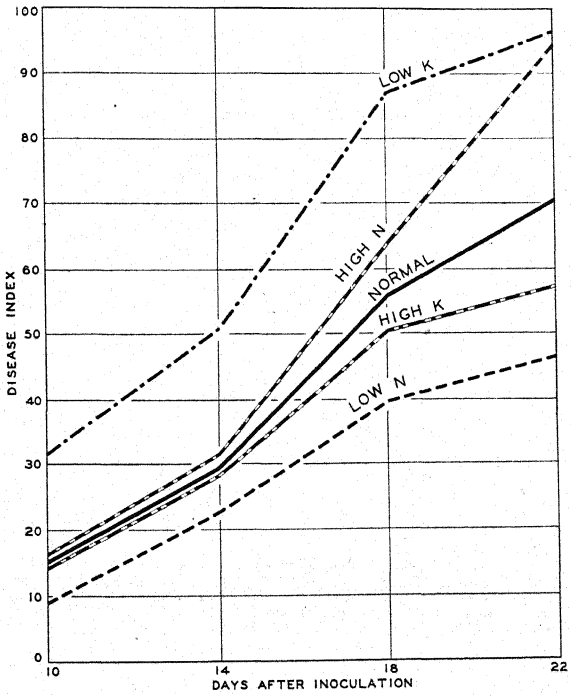


Fig. 3. Relation of the progression of wilt in Bonny Best tomatoes to unbalanced nutrients high and low in potassium or nitrogen.

but the exact manner by which this relationship is effected is still obscure. The most rapid disease development is at the weakest concentration where both host and pathogen are growing slowly. However the suppression of host growth is somewhat greater at this level than the suppression of fungus growth. There was a rather close negative correlation between diffusion pressure deficit of tissue of the host and disease development in the nutrient concentration series. It should be pointed out, however, that when the osmotic concentration of the nutrient solution was kept constant and the level of the potassium or nitrogen varied, about the same extremes in the rate of disease development were secured.

It is of interest to compare the reaction to wilt of susceptible, intermediate resistant and highly resistant tomato hosts with that to cabbage yellows of a similar range of cabbage hosts (Walker and Hooker, 1945a). When the same strain of the pathogen was used, the disease expression of the intermediate resistant tomato varieties followed the same curve pattern in relation to salt concentration as the susceptible varieties but always at lower values. In this regard the relation is the same as noted in cabbage. When single-gene high resistance was present in the tomato host, no disease was produced at any extreme of nutrient concentration or balance used. This was also the case in cabbage. Thus the multiple-gene resistance to *Fusarium* is profoundly influenced by host nutrition in tomato as in cabbage, while single-gene resistance is not so influenced and remains stable through wide environal variation. In field practice this latter type of resistance should, therefore, be much more satisfactory than multiple-gene resistance. This has been found to be the case in cabbage.

There has been opportunity in this investigation to study the effect of host nutrition on disease development in susceptible and intermediate resistant hosts when exposed to virulent and mildly pathogenic strains of the parasite. It was possible to produce the disease more rapidly in an intermediate resistant variety grown in a given nutrient concentration and exposed to the virulent pathogen, than in the susceptible variety grown in the same nutrient concentration and exposed to the mild pathogen. The important point is that wilt development, brought about by either the mild or the virulent strain, had the same relative relation to nutrition even though there was a difference in total disease development during a given period of time.

SUMMARY

The relation of nutrition to *Fusarium* wilt development in infected young tomato plants grown in sand under greenhouse conditions was studied. Solutions of varying concentrations of all elements were used. These were based upon Hoagland and Snyder's (1933) solution (1H), and variations were made by decreasing the total salt concentration to one-tenth (0.1H), to one-half (0.5H), and by increasing the concentration twice (2H) and three times (3H). Disease development, in susceptible or intermediate resistant hosts grown at a temperature optimum for wilt, decreased with an increase in nutrient concentration regardless of the degree of virulence of the pathogen used or the age of the plants within the limits of the studies. Increased nutrient concentration up to the 2H level occasioned

an increased diffusion pressure deficit in cells of uninoculated plants. Growth of both the host alone or the pathogen alone was low in the 0.1H solution, reached a maximum in the 1H nutrient, and decreased with further increase in total salt concentration.

Unbalanced nutrition brought about an alteration of the susceptibility of tomato plants to wilt which was not correlated with total plant growth. Studies on the effect of nutrient balance upon disease development revealed that infected plants receiving a low concentration of potassium or a high concentration of nitrogen showed an increased wilt development. Solutions low in nitrogen or high in potassium brought about a decrease in disease severity.

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INFLUENCE OF TIME OF DAY ON LATEX FLOW FROM CRYPTOSTEGIA GRANDIFLORA [1]

J. T. Curtis and R. Blondeau[2]

CRYPTOSTEGIA GRANDIFLORA R. Br., a climbing semi-shrub of the *Asclepiadaceae*, is one of the many plants whose rubber producing potentialities have been studied as possible sources of natural rubber to supplement that normally obtained from the Para rubber tree, *Hevea braziliensis* (Symontowne, 1943; Viswa Nath, 1943). The investigations here reported represent a portion of the work conducted by the Research Division of the Societe Haitiano-Americaine de Developpement Agricole, in cooperation with the Rubber Development Corporation, an agency of the United States Government. They were carried out in the West Indian republic of Haiti, at Gonaives, on the northeast shore of the Baie de la Gonave.

Cryptostegia was introduced to Gonaives in 1908 as a garden ornamental and has escaped since and become well established over a large portion of the Gonaives Plain, an arid, sea-level, alluvial flat with a high water table. The plant is particularly abundant along water courses and in *Prosopis juliflora Acacia farnesiana* woodlands. It occurs as a hedgerow plant throughout the irrigated farmlands of the plain. A four hundred-acre experimental plantation was established in 1942 and 1943 in the central portion of the plain, about three miles from the town of Gonaives. The experimental results and the weather records included in the present paper were obtained on or near this plantation.

There are two general methods of obtaining rubber-like hydrocarbons from plants. In laticiferous plants, latex may be collected by some system of tapping and the rubber then precipitated from the latex by coagulation, as is done with *Hevea, Castilloa, Manihot, Funtumia* and other genera. The other method for both lacticiferous and non-lacticiferous plants is to harvest the leaves and branches and extract the rubber by mechanical means, as is the case with the guayule bush, *Parthenium,* and the gutta-percha bearing *Pallaquium*. The latex method ordinarily produces hydrocarbons of superior quality because of the lack of contamination of the product with plant debris, such as is commonly the case in mechanical methods. For this reason, and because no successful procedure had been discovered for the mechanical recovery of rubber from *Cryptostegia,* the Gonaives plantation was begun with the idea of developing methods for latex collection.

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The present paper deals with the variations in latex and rubber quantity and quality as influenced by diurnal fluctuations in environmental factors. Tapping of *Hevea* trees usually is carried on only in the early morning, as there is a greatly decreased latex production later in the day (Hauser, 1930). The same is true for the chicle tree, *Achras zapota,* as was shown by Karling (1934), who demonstrated the multiple correlation between atmospheric humidity, trunk diameter and latex flow in this species. The presence of a limited daily optimum period for latex production in *Cryptostegia* would affect greatly the operational routine of a plantation, while considerable differences in latex quality at various hours of the day might necessitate the use of several different factory procedures.

MATERIALS AND METHODS.—The plants used in these investigations were of two types—plantation plants six to twenty-four months old grown from transplanted nursery seedlings, and wild plants of

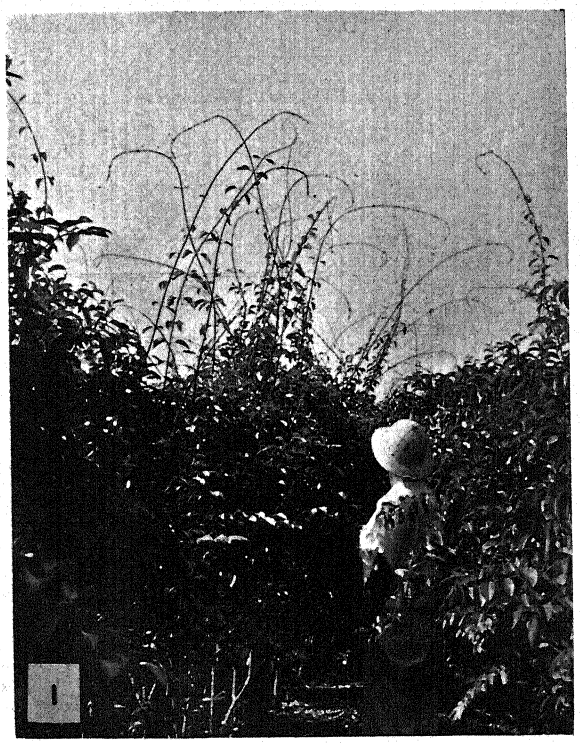


Fig. 1. Photograph of a row of 18-month old plants of *Cryptostegia grandiflora,* showing long, tendril-like vegetative branches.

unknown age (probably ten to twenty years old) spontaneously produced from self-sown seeds. The plantation plants were grown at the rate of 9680 per acre, spaced one foot by one foot in double rows nine feet apart, center to center. The plantation plants were of shrub habit, whereas many of the wild plants were lianas climbing 20 to 60 feet in the overhead trees. *Cryptostegia grandiflora* is characterized by the presence of long, tendril-like, vegetative branches, herein called whips (fig. 1). When the plants are grown in the open, the whips reach a maximum length of 16 to 18 feet and then gradually topple over onto adjacent plants. The whips are essentially leafless in the terminal three or more feet, are succulent when young, and are of very rapid growth, averaging two to three inches a day in length increment. They possess latex tubes in both pith and cortex (Trecul, 1866) and have a marked development of internal phloem at the periphery of the pith. The cross-sectional number of latex tubes is relatively constant throughout the length of a whip, except in the basal internodes where new tubes develop in the secondary phloem. Whips of average vigor have about 3200 latex tubes per cross section, of which 800 are in the pith and 2400 are in the outer phloem and the cortex. The pith tubes average 33.6 μ in diameter while the bark tubes are only 20.0 μ. The total volume of the latex tubes in a normal internode is therefore 0.18 to 0.24 cc., since the internodes in the central portion of a whip average 12 to 16 cm. in length.

Latex collection.—A considerable quantity of latex exudes from a whip when the terminal portion is removed by a sharp knife cut. If a small portion (½ to 3 inches) of the whip is cut off on the following day, further latex appears. This process may be repeated daily until the base of the whip is reached. Such repeated cutting of the whips is the foundation for the large-scale latex collection methods in use on *Cryptostegia* plantations, both in Haiti and in India (Viswa Nath, 1943). The liquid obtained is not entirely the product of the latex vessels since it may contain an undetermined quantity of accessory tissue exudate and, at least under certain conditions, liquid from the xylem tissues as well.

Latex also may be obtained from other organs of the plant. Initial cuts on tips of the divergent fruit follicles give a much greater quantity of latex than is obtained from whips, but continuing production is much lower. Fruit latex is commonly maintained under conditions of higher pressure than that in other parts of the plant (Bangham, 1934). The bark of old trunks is plentifully supplied with latex vessels (Meunier, 1912), and latex of very high rubber content (30 to 40 per cent) may be collected from it by tapping the trunks in the same manner as is done with *Hevea.* Rubber from such latex is superior in physical and chemical properties to that from whips or fruits and is nearly equal to that of *Hevea* (Blondeau and Curtis, 1946). This great variation in the rubber content of latex from different parts of the same plant was recorded first by Jumelle (1901) working in Madagascar with wild plants of the other species in the genus, *Cryptostegia madagascariensis* Bojer, and was confirmed for plantation plants of *C. grandiflora* by Curtis, Blondeau, Hitchcock and Van den Berghe (1943). The rubber hydrocarbon content of dried latex from single plants of *C. grandiflora* in Haiti varied from a low of 49.81 per cent for whip latex to a high of 91.1 per cent for trunk latex. The range for cultivated *C. madagascariensis* in Haiti was considerably greater,

whip latex solids containing only 16.7 per cent rubber hydrocarbon as compared to 96.87 per cent in latex from the bark of old trunks. All trunk latex used in these studies was obtained from old wild plants of *C. grandiflora* growing in the Habitation Dessources, a particularly arid section of the Gonaives Plain with a water table at 36 feet.



Fig. 2. Photograph of an old trunk of *Cryptostegia grandiflora*, showing spiral tapping cuts, latex trough and collection bottle. The tapping knife is at the left.

The latex from whips in these experiments was obtained usually by cutting through the apical end of the youngest mature-length internode with a sharp knife and collecting the latex in a test tube. Randomized groups of 15 to 40 stems of uniform length and caliper (8 to 10 feet long and 3.5 to 4.0 mm. in diameter at the first internode cut) were tapped at specified hours throughout the day. In some experiments, only the first day's latex was used. In others, the cuts were repeated at 24 hour intervals for 7 or 14 days, removing one inch of stem at each cut. Results in all cases were expressed as an average yield per stem per day. In addition to the liquid latex produced, a small plug of coagulated rubber formed on the end of each whip as the latex flow lessened; this was collected on the day following the cut in question and was added to the rubber produced from the latex to give the total rubber yield per whip. Latex in some experiments was collected from groups or bundles of whips cut simultaneously in a specially constructed holder. In these cases, the whips were not selected for uniformity. Errors due to whip size variation were eliminated by using large numbers of whips at each hour, usually four or five hundred. The latex from the end of the cut bundle was allowed to drip through a funnel into a milk bottle.

Latex from trunks was obtained by making a spiral cut in the bark over an arc of one half the circumference with a modified *Hevea* knife (fig. 2). The cut was made nearly to the cambium. The plants used in the experiments had been tapped previously at weekly intervals for nine weeks. Fruit latex from plantation plants was collected from the first day cut of both follicles of each of twenty fruits at each hour of the day. No plugs were collected on the fruits, as they were too small to be of significance.

Latex in all experiments here reported was coagulated by the addition of 0.1 per cent Nacconal, a proprietary synthetic detergent of the sulfonated hydrocarbon type (Curtis, Blondeau, Ford and Seifriz, 1943). Coagula were rolled in a tight rubber sheeting mill and dried at 70°C. Total solids were determined by drying latex aliquots at 70°C., followed by further drying over calcium chloride to constant weight. Acetone and water extractions of coagula and total solids followed customary analytical methods, with 16-hour extraction periods in ASTM extractors for acetone and in Soxhlet extractors for water. Acidity was determined by a Beckman glass electrode within ten minutes after

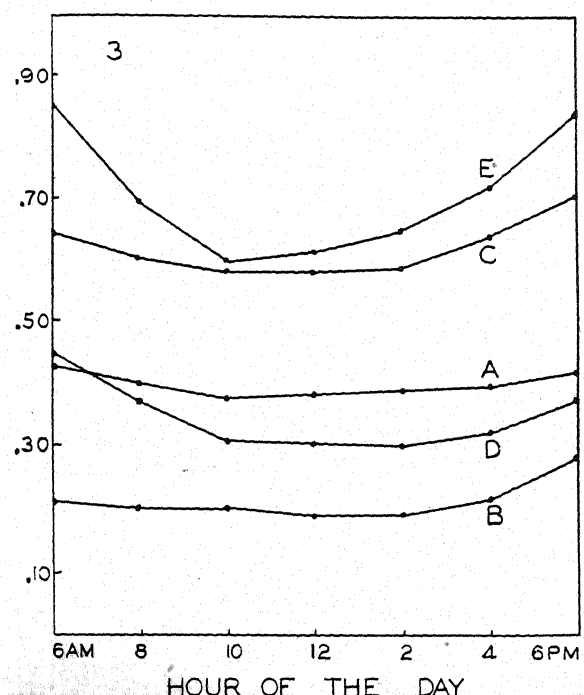


Fig. 3. Graph of first day yields from whip-tapping plotted against hour of day. The lines represent average yield per whip of coagulum rubber (A), plug rubber (B), total rubber (C), latex (D) and total solids (E). Figures on the ordinate are decigrams for rubber and solids and cubic centimeters for latex.

latex collection. Temperature corrections were applied to the electrode readings to compensate for the wide variation in latex temperatures at various hours of the day.

Continuous records of air temperature and relative humidity were obtained with a Friez hygrothermograph. No apparatus was available for determination of light intensity, but all experiments here reported were conducted under conditions of full sun for the entire day.

EXPERIMENTAL RESULTS.—*Whip latex.*—The yield of latex and rubber was determined for plants of different ages and at different seasons during the period from January, 1943, to January, 1945. The absolute magnitude of the yields varied with both plant age and season, as will be reported in detail elsewhere, but the trend at different hours of the day remained nearly constant in all experiments. Figure 3 presents the results obtained in a typical trial made in January, 1944. Only first day yields are shown. All measures of yield showed a minimum value sometime between 10 a.m. and 2 p.m. Latex and coagulum values were higher in the early morning hours than in the late afternoon, whereas the total rubber produced was greatest in late afternoon, due to the rise in plug yields at those hours.

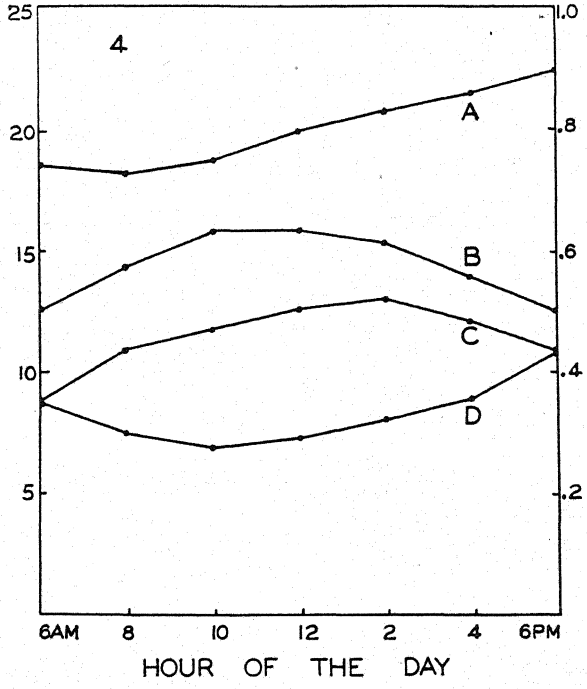


Fig. 4. Graph of changes in proportions of whip latex components at various hours of the day. Total solids (A), dry rubber content (C), and total non-rubber serum solids (D) are expressed as a percentage of the latex weight on the left ordinate. Figures for the ratio of rubber to total solids (B) are given on the right ordinate.

Figure 4 shows the variation in total solids, dry rubber content, and total non-rubber serum solids expressed as a percentage of the latex, and also the dry rubber content expressed as a percentage of total solids. The highest rubber content was reached at 2 p.m., but the total solid matter increased gradually to 6 p.m. as a result of the increase in serum solids. The high serum solids concentration was maintained during the night, with an abrupt reduction in the early morning.

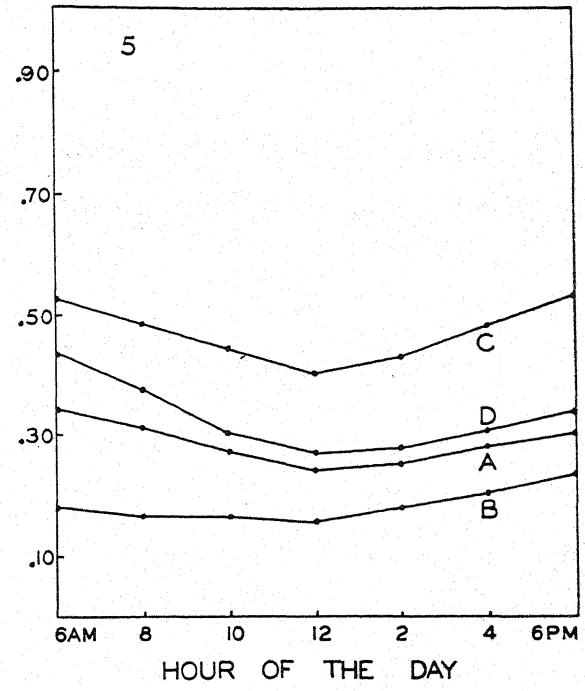


Fig. 5. Graph of average whip yield of six experiments from January, 1943, to July, 1944. The lines represent average yield per whip of coagulum rubber (A), plug rubber (B), total rubber (C), and latex (D). Figures on the ordinate are decigrams for rubber and cubic centimeters for latex.

Similar results were obtained in all experiments. Data obtained by averaging the results of six experiments in January, February and September, 1943, and January, March and July, 1944, are presented in figure 5. The average daily march of temperature, relative humidity and vapor pressure deficit for the periods of all experiments is portrayed in figure 6. Relative humidity values reached their minimum before the maximum temperature was reached. The increase in humidity with increasing temperature in the early afternoon was caused by the daily advent of a moist sea breeze at that time. Full recovery of early morning values for all three environal factors usually was attained by midnight with relatively constant maintenance thereafter until sunrise.

Changes in the chemical composition of both latex and rubber coagulum during the day were investigated by making determinations of the amounts of acetone-soluble and water-soluble substances present. Results of a typical experiment are given in table 1. Fluctuations in latex, coagulum and

TABLE 1. *Variation in latex yield, composition and pH. Latex from 400 whips at each hour, March 9, 1944. Plantation plants, 12 months old.*

Character	6 a.m.	8 a.m.	10 a.m.	12 noon	2 p.m.	4 p.m.	6 p.m.
Latex per whip (cc.)	0.61	0.43	0.28	0.20	0.23	0.29	0.35
Coagulum per whip (mg.)	30	21	15	11	12	15	18
Acetone extract of coagulum (%)	11.8	10.8	10.5	10.7	10.5	11.0	11.8
Water extract of coagulum (%)	0.7	1.0	1.2	1.2	0.8	0.5	0.4
Total extract of coagulum (%)	12.5	11.8	11.7	11.9	11.3	11.5	12.2
Latex pH	5.31	5.08	5.00	4.93	4.92	5.02	5.24

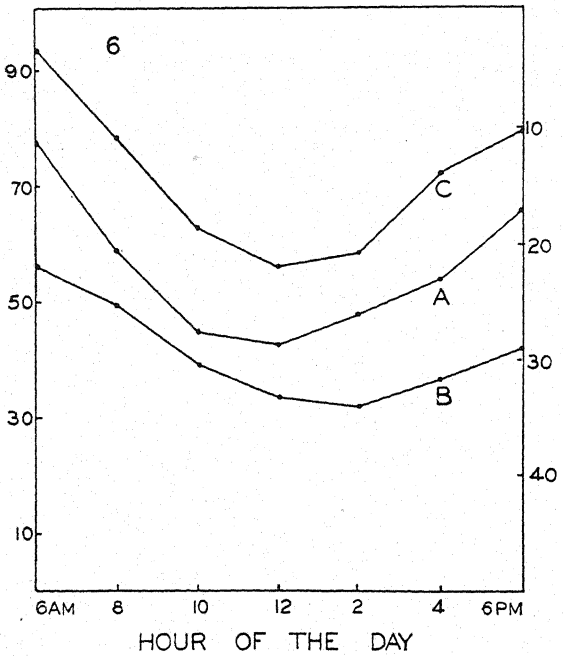


Fig. 6. Graph of the average daily march of relative humidity (A), temperature (B) and vapor pressure deficit (C) for the periods of all experiments. Left ordinate is relative humidity in per cent; right ordinate is temperature in degrees Centigrade and vapor pressure deficit in millimeters of mercury, with scale values increasing from top to bottom.

total solids yields during the day in this experiment were similar to those reported above. The amount of acetone-soluble substances in the coagulum varied only slightly during the day with minimum values at midday hours. Water-soluble substances were present only in very low amounts, but the maximum value was reached from 10 to 12 a.m.

Table 1 also gives the figures for latex pH at the various hours. Greatest acidity was attained between 12 noon and 2 p.m. concurrently with minimum latex and rubber yields. While these pH changes are slight, they are nevertheless real and have been obtained repeatedly.

Fruit latex.—Latex and dry rubber content expressed in milligrams per fruit, and dry rubber content as a percentage of the latex are shown in table 2, from data collected in September, 1944, on fruits of 18-months-old grafted plantation plants (Clone No. G-5). Maximum latex and rubber yields were obtained in early morning. Both reached low values by noon, but only the latex yields showed a tendency to recover in the late afternoon.

Trunk latex.—A quite different response to the diurnal change in environment was shown by the latex obtained by trunk tapping. Maximum yields of both latex and rubber occurred between 9 a.m. and 10 a.m., with a low point shortly after noon. The greatest dry rubber content occurred simultaneously with the greatest latex yield whereas the converse was true with whip latex. Average values for latex, dry rubber and total solids per tree are shown in table 3, along with acetone and water extracts of total solids, acetone extracts of coagulum rubber, and latex pH. Perhaps the most significant changes were exhibited by the water-soluble materials in the total solids. Such materials decreased from more than 10 per cent of the total in the early morning hours to less than 5 per cent in the late afternoon. Another noteworthy feature of the trunk latex was its relatively great stability with regard to pH changes. What little change did take place, however, resulted in the lowest pH at the time of maximum latex and rubber yield, which is again contrary to the findings in whip latex, where decreased yield was accompanied by lowered pH values.

DISCUSSION.—Yields of latex and rubber from whips, fruits, and trunks of *Cryptostegia grandi-*

TABLE 2. *Variation in yield of latex and coagulum from fruits. Average values per fruit for 20 fruits at each hour, September 20, 1944. Plantation plants, 18 months old.*

Character	6 a.m.	8 a.m.	10 a.m.	12 noon	2 p.m.	4 p.m.	6 p.m.
Latex (cc.)	1.82	1.59	1.42	1.38	1.29	1.38	1.45
Coagulum (mg.)	345	318	297	296	292	293	292
Air temperature (°C.)	20.6	23.9	30.6	33.3	32.8	31.1	27.8
Relative humidity (%)	100	64	55	43	48	57	83
Vapor pressure deficit (mm. Hg.)	0.0	8.0	14.8	21.9	19.4	14.6	4.7

TABLE 3. *Variation in trunk latex yield, composition, and pH. Average values for latex from 50 trees at each hour, March 13, 1944. Wild plants ten years or more old.*

Character	7:20 a.m.	8:10 a.m.	9:40 a.m.	11:00 a.m.	12:35 p.m.	3:00 p.m.	4:25 p.m.
Latex per trunk (cc.)	1.20	1.58	1.88	0.97	0.45	0.62	0.61
Coagulum per trunk (mg.)	312	411	635	291	120	197	180
Dry rubber content (% of latex)	26.16	26.00	33.79	30.00	26.68	31.60	29.50
Total solids (% of latex)	35.90	35.05	43.20	39.90	37.54	40.20	40.90
Latex pH	5.31	5.29	5.24	5.28	5.32	5.35	5.35
Acetone extract of coagulum (%)	7.5	7.6	7.8	7.5	7.1	7.2	7.0
Acetone extract of total solids (%)	5.60	5.55	5.72	5.86	5.55	5.40	5.03
Water extract of total solids (%)	10.0	10.1	10.2	9.6	7.4	5.8	4.9
Air temperature (°C.)	23.9	25.3	30.0	31.4	32.2	32.8	29.4
Relative humidity (%)	82	75	58	56	55	59	57
Vapor pressure deficit (mm. Hg.)	4.1	6.1	14.0	15.2	16.0	14.8	16.0

flora reach a minimum value around midday. Such atmospheric factors as relative humidity, temperature and vapor pressure deficit attain their extreme values at this time also. There is a very good negative correlation between vapor pressure deficit and yield of latex, coagulum and total rubber, particularly from whips. Vapor pressure deficit figures are here taken to be the best available measure of relative evaporating power of the air, although it is realized that they do not parallel evaporation rates from open water surfaces under all conditions. The inverse relationship between yield and potential evaporation demonstrated by the above data is substantiated by the observation that total latex production from the 400-acre experimental plantation is always low during those periods when a local *sirocco* induces conditions of extreme aridity. During midday hours the exposed leaf blades of *Cryptostegia* are curled outward and the leaves droop at the petiole in a semi-wilted condition, regardless of the amount of soil water available. This condition is particularly noticeable when vapor pressure deficit figures exceed 20 mm. Hg. The rate of evaporation from the leaves is greater than the rate of water replacement from the roots and this high transpiration rate apparently causes the withdrawal of some water from the latex vessels and accessory tissues, as is evidenced by the greatly lowered total flow of latex. Another indication of lowered internal hydrostatic pressures is the reduction in total latex dripping time at noon as contrasted with either early morning or late afternoon values. Thus, in several experiments, the average time necessary to collect 98 per cent of the total latex from a given cut was 5.0 minutes at 6 a.m., 2.9 minutes at 12 a.m., and 4.2 minutes at 5:30 p.m. This reduction in flow occurs even though the lower viscosity of the latex at midday temperatures might be expected to increase the quantity of exudate.

Values for dry rubber content as a percentage of the latex increase to a maximum at midday and then decrease, roughly paralleling the changes in vapor pressure deficit. The absence of any known mechanism whereby the absolute rubber content could decrease through digestion and metabolic utilization makes it seem probable that the apparent change in the afternoon is due to variation in the amounts of other constituents, rather than the rubber itself.

The continued rise in total solids in the late afternoon against an increasing cell hydration gradient would indicate that the accessory tissue exudate and possibly the latex itself contained an increased concentration of non-rubber substances, which may have resulted from photosynthetic activity during the day. These unknown substances appear to be readily utilized, either by translocation or local metabolism, since they return to a low concentration by 8 a.m. (fig. 4).

The yield of trunk latex at various hours of the day differs from that of whips primarily in that the maximum yield is obtained between 9 a.m. and 10 a.m. This fact is well known to the local collectors of wild rubber who tap the trees only between 8 and 11 a.m. No completely adequate explanation for this lack of correlation between maximum plant turgor and maximum latex flow can be given, except for the observation that the trunk latex is very viscous and that the viscosity is sensitive to temperature changes.

The coincidence of the greatest content of both rubber and serum solids with maximum latex flow is also different from the situation in whips where high rubber content was associated with low latex volume. These differences may be correlated with the lack of accessory tissue exudate in the bark but the exact nature of the relationship is unknown.

The diurnal changes in latex and rubber production from fruits are intermediate between those of whips and trunks. Latex volume and latex dry rubber content follow the same pattern as in the case of whips, whereas actual weights of rubber and total solids per fruit are more nearly like those of trunks, being highest in early morning and lowest in the afternoon. The concurrence of high latex yield with high rubber and solids yield is also similar to the situation in trunks. The amount of rubber per unit volume of fruit latex (20 per cent) is in-

termediate between the low value of whips (12 per cent) and the high value of trunks (33 per cent).

From the standpoint of plantation practice, these findings in regard to yield at various hours of the day must be interpreted in the light of the collection method in use. If the trunk tapping method is employed, then it appears obvious that morning tapping only is the proper procedure since the average afternoon yield is less than one half that of the morning. For whip tapping, the time of day is not so important, since the lowest hourly yield of total rubber is nearly four fifths that of the maximum yield, and, in addition, the late afternoon yields equal or exceed those of early morning. Fruit tapping also may be conducted throughout the day, since the low value is only 15 per cent less than the maximum. In field practice, the current procedure for whips and fruits is to tap from sunrise to 11 a.m. and from 1 p.m. to 5 p.m. The two hour midday rest period thus eliminates the hours of lowest yield and raises the daily average yield while at the same time it allows a sufficiently long work day to enable the tappers to complete a relatively large daily task. This last condition is essential if rubber at a reasonable price is to be obtained by whip tapping, due to the very low yield per whip and the consequent necessity of cutting a large number of whips per man day.

Quality changes in the rubber as expressed by acetone and water extracts are so small as to be negligible. In general, the acetone-soluble substances in the rubber are more abundant at times of greatest yield in both whips and trunks but the differences are too slight to affect factory procedure or final rubber quality.

SUMMARY

Studies of latex and rubber production from various plant parts of *Cryptostegia grandiflora* at different hours of the day were conducted on both wild and plantation plants under semi-desert conditions at Gonaives, Haiti. Minimum yields of latex and rubber were obtained in all cases during midday hours. Yields of rubber from the bark of old trunks and from young fruits continued low throughout the afternoon, whereas yields from the whip-like vegetative branches returned in the late afternoon to a point equalling or exceeding that of early morning.

The time of minimum yield was correlated with the time of greatest atmospheric evaporating power as measured by the vapor pressure deficit. Latex composition changed considerably throughout the day, the rubber fraction of the total solids varying from 50 per cent in the early morning to nearly 65 per cent at noon in the case of whip latex. Latex acidity also varied, being most acid in the midday hours and least acid in the early morning and late afternoon. Only minor diurnal differences in quality of the rubber obtained by latex coagulation were found.

From the standpoint of plantation practice, it appeared that only morning tapping should be used for trunk latex collection, but that whip latex and fruit latex could be collected throughout the day, with provision for a relatively long noon-time rest period.

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PLANT MICROFOSSILS FROM A FORT UNION COAL OF MONTANA [1]

Leonard R. Wilson and Ruth M. Webster

Fossil spores and pollen are abundant in coals occurring in the Fort Union series near Red Lodge, Carbon County, Montana. Previous to this discovery only a single species of fossil spore (Miner, 1935) and no pollen grains were known from the Paleocene rocks. In North America, very few of the extensive Tertiary coal deposits have been explored for plant microfossils, although such study has great possibilities for the advancement of knowledge concerning the composition and stratigraphy of coal.

The coal field in the vicinity of Red Lodge and Bear Creek, Montana, is extensive. According to Dr. Erling Dorf (personal written communication), "These coals are very high in the Fort Union series, are definitely post-Lebo, and probably post-Tongue River as now restricted (see Wood *et al.*, Bull. Geol. Soc. Amer., vol. 52, plate 1, 1941)." Woodruff (1909) has shown that there is a total of more than eighty-one feet of coal in the numerous seams. These occur through an 825-foot section of the Fort Union series. The coals range from smuts to seams twelve feet in thickness. There is a general thinning of the seams toward the southwest and thickening toward the northwest. The areal distribution of the seams indicates that they are more extensive than local lenticular deposits.

The structure of the coal field is an eroded monocline which dips southwestward from the Pryor Mountains. At the foot of the Beartooth Range the monocline is terminated by a fault whose throw is several thousand feet. The dip of the beds in the vicinity of Red Lodge is 18°, but south of Bear Creek the dip is much less. Minor undulations occur in the beds, and a small dome is present near the latter town.

In 1940 and 1941 twenty-seven channel samples of coal were collected from seams one to eight inclusive. The seams are numbered from the top down. Maceration of the samples has revealed the presence of spores and pollen in varying degrees of abundance. Those coals composed mostly of modified woody tissues contain very few spores or pollen and have proved unsatisfactory for statistical correlation.

This paper describes the fossil spores and pollen from one deposit, the Kolarich Mine. This mine is located in Section 25, Township 7 S., Range 20 E. It is approximately two miles east of Red Lodge. Some uncertainty exists concerning the position of this coal seam. Opinions of local residents are divided as to whether the Kolarich Mine is exploiting seam No. 1 or No. 3. No conclusive evidence has yet been secured from microfossil studies that will solve the problem.

The coal sample was secured from the wall several yards from the entrance of the drift mine. The rock section in the Kolarich Mine consists of (1) a gray undershale, (2) coal, 6 feet, (3) shale parting, 6 inches, (4) coal, 6 inches, (5) gray roof shale.

Methods.—Tertiary coals require various maceration treatments because they range widely in composition and structure. Since the Kolarich coal is both woody and close to sub-bituminous rank, it was necessary to use Schultz's solution for maceration. Pieces of coal were selected that did not contain visible woody structure. These were broken into pieces approximately one-half centimeter in diameter and covered with Schultz's solution for 24 hours. In the course of the maceration the mass was frequently stirred. This treatment was followed by washing with water, and decantation, until the odor of Schultz's solution was no longer apparent. The coal was then covered with ammonium hydroxide for another 24 hours. Again the material was washed and decanted until the odor of the ammonium hydroxide disappeared. The remaining sediment was examined for megaspores, but none were found. The finer sediment was stained with safranin, washed, centrifuged and transferred to glycerine jelly. Permanent slides were made from this preparation. All specimens are in the collection of the senior author.

Thallophyta

Fungi

Brachysporium sp. (?) (fig. 1).

Fungus spores are frequent in the Kolarich coal but are few in types. Figure 1 illustrates the most abundant form. The structure and size of these are fairly constant. The spores are composed of one to several cells and range from 30 to 76 microns in length, depending upon the number of cells in the spore. In width the widest cell ranges from 28 to 48 microns. The largest cell, usually the terminal, is darker brown than the others; it is frequently black and opaque. The other cells are usually translucent and brown. The "cross walls" are two-lobed, each lobe being triangular in view. The surface of the spore is smooth to slightly roughened. A slide of the fossils was sent to Dr. J. C. Gilman who has kindly furnished the following comment concerning the identity of the material. "Boedijn (Bull. Jard. Bot. Buitenzorg (Sec. III) 13: 12–134, 1933) figures a spore of this type for *Brachysporium obovatum* (Beck.) Sacc. Saccardo gives the spore size for this species as 25 × 13 microns, and lists under synonymy *Helminthosporium obovatum* Berk. and

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The authors wish to express their appreciation to Dr. Erling Dorf for information concerning the age of the Kolarich coal, to Dr. J. C. Gilman for examining the fungus spores, and to Dr. Theodor Just for reading the manuscript and checking the construction of the new names and combinations. For a grant-in-aid, the authors are indebted to the American Association for the Advancement of Science.

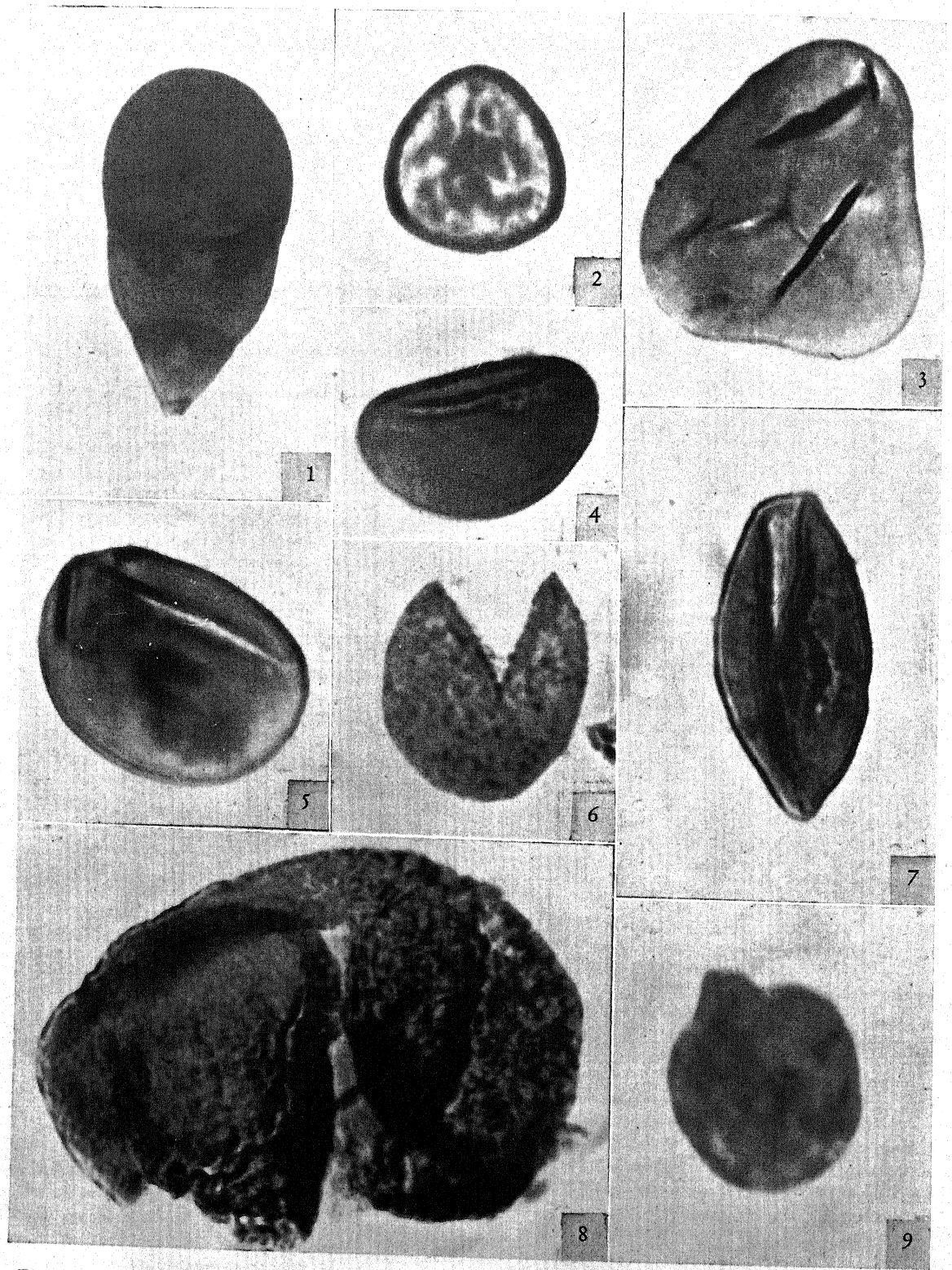


Fig. 1-9.—Fig. 1. *Brachysporium* (?) sp. 48 × 76 microns.—Fig. 2. *Sphagnum antiquasporites* sp. nov. (Holotype) 24 microns.—Fig. 3. *Deltoidospora diaphana* sp. nov. (Holotype) 45 microns.—Fig. 4. *Laevigato-sporites gracilis* sp. nov. (Holotype) 18 × 30 microns.—Fig. 5. *Laevigato-sporites ovatus* sp. nov. (Holotype) 27 × 37.5 microns.—Fig. 6. *Taxodium hiatipites* Wodehouse 27 × 30 microns.—Fig. 7. *Cycadopites follicularis* sp. nov. (Holo-

Monotospora biseptata Peck. It would not be *Helminthosporium* in a modern sense, and as *Monotospora* has been used by Corda and Saccardo in different senses, I think *Brachysporium* would be the nearest genus of the Imperfecti for your spore. *B. obovatum* was first described from decaying wood. There is another, but to my mind a more remote possibility, that this spore might be the teleutospore stage of a rust (Uredineae). There are few rust teleutospores with 3-cells however. A third suggestion also more remote than the first comes from the lobed shadows below each of the 'cross walls.' What makes the separation of the lobes? If this division was interpreted as caused by a median longitudinal wall, then the closest Imperfect genus would probably be *Macrosporium* which contains many species that are common on decaying vegetation."

Until more information can be gathered from the fossils, no specific name will be applied to this spore.

BRYOPHYTA

Sphagnaceae

SPHAGNUM **antiquasporites** sp. nov. (fig. 2).

Spores trilete, oblately flattened, circular to slightly triangular in equatorial view. Diameter 23–25 microns; wall 1–1.5 microns thick, smooth, translucent; trilete rays simple, 5–6 microns long. Holotype: Slide 352C-2.

The spores of *S. antiquasporites* are structurally similar to those of modern species and indistinguishable from most fossil forms observed in peat deposits. *Sphagnum* spores are not frequent in any Tertiary coals yet examined, which is surprising, considering that they are very abundant in Quaternary peats, and in at least one Cretaceous coal of the Rocky Mountain Region. The latter deposit is located at the Goose Wing Mine, Gros Ventre Mountains, Wyoming. There the identity of the spores is further strengthened by the association of *Sphagnum* "leaf" tissue. Kirchheimer (1934), Rudolph (1935), and Thiergart (1937) have described the occurrence of *Sphagnum* spores in German Tertiary coals.

PTERIDOPHYTA

DELTOIDOSPORA **diaphana** sp. nov. (fig. 3).

Spores trilete, oblately flattened, triangular to subangular in equatorial view; diameter 42–46 microns; wall 1–2 microns thick; smooth, translucent, frequently with taper-point folds; trilete rays simple, 12–15 microns long. Holotype: Slide 345C-1.

This fossil occurs rarely.

The genus *Deltoidospora* was originally described by Miner (1935) for small unassigned deltoid or sub-deltoid spores of the type that is commonly found associated with many Mesozoic ferns such as *Gleichenites, Gleicheniopsis, Laccopteris* and others.

LAEVIGATO-SPORITES **ovatus** sp. nov. (fig. 5).

Spores monolete; broadly bean-shaped; length 33–39 microns, width 22–30 microns; wall smooth, translucent, 1.5–2 microns thick; monolete suture simple. Holotype: Slide 345C-2.

This is the most abundant microfossil in the Kolarich coal.

LAEVIGATO-SPORITES **gracilis** sp. nov. (fig. 4).

Spores monolete; narrowly bean-shaped; length 27–30 microns, width 16–19 microns; wall smooth, translucent, 1–2 microns thick; monolete suture simple. Holotype: Slide 345C-2.

Not as frequent as the former species, but nevertheless an important fossil.

The inclusion of the above two species of spores in the genus *Laevigato-sporites* is not entirely satisfactory, for there is little doubt that these spores belong to either *Thelypteris, Asplenium, Athyrium, Aspidium,* or *Blechnum,* and probably should have greater phylogenetic recognition than the name implies. Certain species within these genera of modern ferns have spores that are either smooth or shed their perispores very easily. The remaining endospore is smooth. Such a condition makes it impossible to assign with certainty the smooth-walled spore to an existing genus. Three species of *Aspidium* are described for the Fort Union series, but upon that evidence alone it is not possible to assign the above fern spores to *Aspidium.*

The genus *Laevigato-sporites* was first used by German paleontologists for Paleozoic spores, but similar fossils occur throughout the American Mesozoic and Tertiary coals. Since close generic identification of smooth-walled fern spores is not possible, it seems necessary to extend the genus *Laevigato-sporites* to include fossils in younger rocks. Certain morphological types of spores and pollen are definitely restricted to short geological ranges, but the *Laevigato-sporites* type exists from the late Paleozoic to the present. Wodehouse (1933) has suggested the creation of genera indicating the degree of certainty in phylogenetic relationship. The roots of the names have their origin in a genus or family name to which the fossils appear to have closest relation. A similar treatment might be adopted for the two spores described above, since they probably belong to the family Polypodiaceae. The establishment of a genus that would include all spores of the Polypodiaceae would encounter the difficulty of having both trilete and monolete spores within its boundaries, since that family has both forms. The inclusion of the trilete and monolete forms in the genus would cause considerable confusion since other families of ferns have more trilete spores than Polypodiaceae. The assignment of all isolated Mesozoic and Cenozoic smooth trilete spores to the family Polypodiaceae would be an unfortunate and erroneous procedure. For the present at least, the writers are recognizing in the Ter-

type) 18 × 39 microns.—Fig. 8. *Picea grandivescipites* Wodehouse 63 × 93 microns.—Fig. 9. *Sequoia lapillipites* sp. nov. (Holotype) 26 × 31 microns.

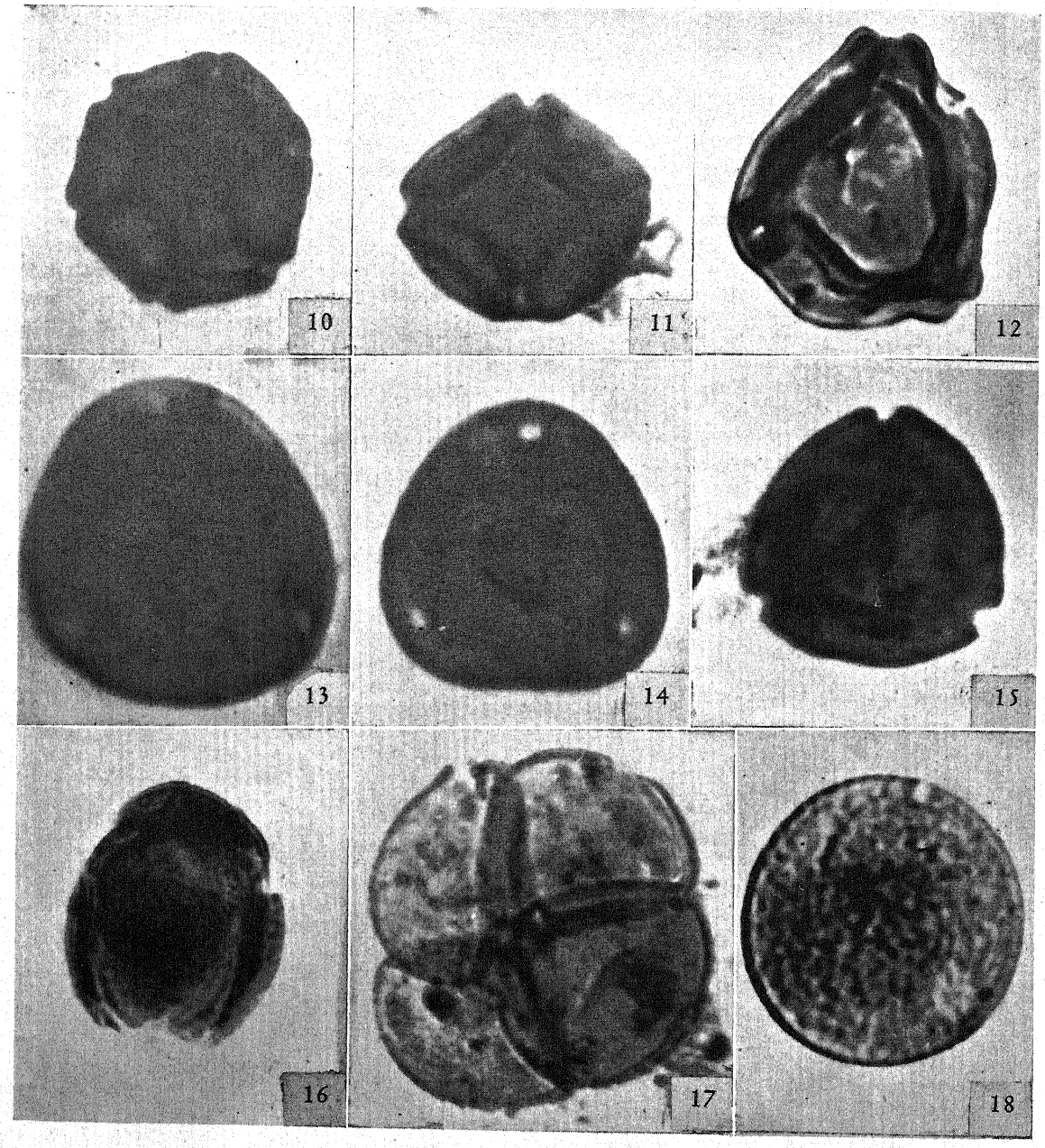


Fig. 10–18.—Fig. 10–11. *Alnus speciipites* Wodehouse 27, 34 microns.—Fig. 12. *Betula claripites* Wodehouse 33 microns.—Fig. 13. *Carya viridi-fluminipites* (Wodehouse) Wilson and Webster, comb. nov. 37 microns.—Fig. 14. *Carya veripites* sp. nov. (Holotype) 33 microns.—Fig. 15. *Momipites coryloides* Wodehouse 28 microns.—Fig. 16. *Quercus priscipites* sp. nov. (Holotype) 24 × 30 microns.—Fig. 17. *Typha latifolipites* sp. nov. (Holotype) 50 microns.—Fig. 18. *Sparganium globipites* sp. nov. (Holotype) 30 microns.

tiary the existence of *Laevigato-sporites* as amended by Schopf, Wilson, and Bentall (1944).

GYMNOSPERMAE

Cycadaceae

CYCADOPITES **follicularis** sp. nov. (fig. 7).

Ellipsoidal; approximately twice as long as wide; length 39–42 microns; width 18–21 microns; furrow extending total length of grain, open at ends, usually closed in the middle by furrow edges overlapping in shrinkage; surface smooth, wall 1.5 microns thick, translucent. Holotype: Slide 364C-1.

The genus *Cycadopites* was originally described by Wodehouse (1933) from the Green River oil shales of Colorado. Wodehouse did not attempt to separate the numerous forms which he observed. In the Kolarich coal specimens that fit the description of *Cycadopites* are abundant, and since they

are nearly uniform in size and similar in structure, they might be considered as belonging to a single species.

Abietineae

PICEA GRANDIVESCIPITES Wodehouse, 1933 (fig. 8).

Specimens of *Picea* pollen in the Kolarich coal are not abundant and when found are usually badly shrunken and folded. This state of preservation does not permit critical comparison, but the dorsiventrally flattened shape of the grain, the pointed appearance of the bladders and the almost straight edges of the bladders on the proximal side make the generic determination certain. The diameter range of *P. grandivescipites* given by Wodehouse is 70–85 microns. The tube cells of the specimens observed in the Kolarich coal are 68–70 microns in longest diameter. Wodehouse based his species on six fairly well preserved pollen grains. He notes that they show some variation in bladder characteristics and that these six specimens may represent two or three natural species. The Kolarich Mine specimens may also represent more than one species, but until an abundance of *Picea* pollen is discovered in the early Tertiary coals and critically studied, it appears desirable to consider the specimens as belonging to *P. grandivescipites* as now described.

Taxodineae

SEQUOIA **lapillipites** sp. nov. (fig. 9).

Grains spheroidal, diameter 27–29 microns; germ pore projecting, conical, somewhat asymmetrical, 8 microns long, 9 microns wide at base; exine smooth, 1–1.5 microns thick. Holotype: Slide 367C-1.

Pollen fossils of *Sequoia* are rare in the Kolarich coal. The two grains that were found are very similar to those of modern species with the exception that the conical papilla of each ends abruptly without the bent tip common to modern *Sequoia*. However, it is highly probable that the tips have been broken off. When fresh *Sequoia* pollen is prepared for study by boiling in acetic anhydride-sulphuric acid, the bent tips are frequently modified, and the papilla present the same appearance as these fossils. Such papillae are also found in the pollen of *Cryptomeria*, an eastern Asiatic tree, but the widespread distribution of *Sequoia* leaf and wood fossils in this region throughout the Tertiary makes it reasonable to consider these fossils as belonging to that genus.

TAXODIUM HIATIPITES Wodehouse, 1933 (fig. 6).

This is the most common gymnosperm encountered in the Fort Union coal. The specimens compare perfectly with the description given for the Green River species. As in the Green River shale, *T. hiatipites* is exceedingly abundant. This species seems to have a very wide range, for it has been observed by the authors in coals of the Frontier formation of western Wyoming, and in the Eocene coal of Wamsutter, Wyoming. In Germany a similar form described as *Pollenites hiatus* occurs in the Eocene, Miocene, and Pliocene coals (Potonie, 1934; Potonie and Venitz, 1934; Wolff, 1934). One macrofossil species, *Taxodium occidentale* Newberry, is listed by Knowlton (1919) as common in the Fort Union series. It is not possible to state that *T. hiatipites* is the pollen of *T. occidentale*, but it is of interest to note that leaves and small branches of the latter were observed in abundance on the roofs of the Smith and Foster mines at Washoe and Bear Creek respectively. These two mines are exploiting seam No. 3, as might also be the Kolarich Mine. Until the two species are found in organic connection, their status must remain as at present.

ANGIOSPERMAE

Betulaceae

ALNUS SPECIPITES Wodehouse, 1933 (fig. 10, 11).

A fairly common component in the Kolarich coal. The most abundant form has four pores, but specimens with five pores are frequent and two specimens with six pores have been found. Kirchheimer (1932) has pointed out that certain *Alnus* grains from the brown coals of Germany with four or six pores are considerably smaller in size than others with four or five pores. A distinction in size of the four-pored forms has been noted in the Fort Union deposit, and the two six-pored specimens are several microns smaller than certain four- and five-pored specimens, but the results are not conclusive and until further evidence is available, no specific distinction is being made in the *Alnus* pollen grains.

Alnus serrata Newberry, a leaf fossil, occurs in the Fort Union formation of Yellowstone National Park.

BETULA CLARIPITES Wodehouse, 1933 (fig. 12).

The fossils here assigned to *B. claripites* fit Wodehouse's description except that one specimen is approximately three microns larger than the largest dimension given. The pore structure of the Kolarich specimens is distinctly that of modern *Betula*, and the size is within the limits of modern species.

This fossil is infrequent in the Kolarich coal, which is somewhat surprising since *Betula* is widely distributed in the Tertiary, and four species of leaves have been described from the Fort Union of Montana.

Urticaceae

MOMIPITES CORYLOIDES Wodehouse, 1933 (fig. 15).

Most of the fossils assigned to this species compare well with the type specimen in the Green River oil shales described by Wodehouse (1933). Further study of size frequency may reveal that two or possibly three species are present in a complex. Their abundance makes them one of the characteristic fossils of the Kolarich coal. This abundance is also true of the oil shale from which the species was originally described. According to Wodehouse, *Momipites* is of problematic status since it also closely resembles *Corylus* and *Engelhardtia*. From the latter it is separated by its larger size. Agree-

ment seems to be closest with *Momisia*. Whatever its true affinity, the plant was of considerable importance in the Tertiary of the Rocky Mountain states. A similar form has been reported from the Eocene of Germany (Kirchheimer, 1932) and of Scotland (Simpson, 1936). Kirchheimer suggests affinity with *Corylus, Ostrya,* or *Myrica,* and Simpson describes the Scottish fossils as belonging to *Corylus*.

Juglandaceae

Carya viridi-fluminipites (Wodehouse) Wilson and Webster, comb. nov. (fig. 13).

Hicoria viridi-fluminipites Wodehouse, 1933. Tertiary pollen—II. The oil shales of the Green River formation. Bull. Torr. Bot. Club 60: 503 (fig. 29).

Many hickory fossils have been described under the name of *Hicoria* Raf. and it may be desirable on this account to continue the practice. However, since the fossils represent a modern genus for which the Committee on the Conservation of Names at the International Congress in Vienna (Briquet, 1935, p. 94) voted to conserve the name *Carya,* it appears pertinent that *Carya* be used. The authors are following the practice of Schopf, Wilson, and Bentall (1944) who have shown the desirability of adhering to the International Rules of Nomenclature as one of the guiding principles in spore and pollen paleontology.

The above species is a common fossil in the Kolarich coal.

CARYA **veripites** sp. nov. (fig. 14).

Grains circular to slightly triangular in equatorial view, oblately flattened; diameter 27–33 microns; germ pores three, round to broadly elliptical in shape, 3–4.5 microns long, all in one hemisphere, placed 3–4.5 microns from the equator; exine smooth, translucent, approximately 1.5 microns thick near equator; polar areas apparently thinner and sometimes corroded or broken, resulting in an irregularly modified area near the center of the grain. Holotype: Slide 355C-2.

This species is not as frequent as the former but is one of the important fossils in the flora. It differs from *C. virdi-fluminipites* in being three to nine microns smaller and more angular; the pores are about 1.5 microns larger, and farther from the equator by approximately 1.5 to 3 microns.

Three species of *Carya* (*Hicoria*) leaves are described from the Fort Union series of the region (Knowlton, 1919).

Fagaceae

QUERCUS **priscipites** sp. nov. (fig. 16).

Grains spheroidal; tricolpate, furrows meridionally arranged, taper pointed, membranes smooth; exine 1.5 microns thick, coarsely warty granular; diameter 24–30 microns. Holotype: Slide 368C–1.

Rare in the Kolarich coal. The specimen used in the illustration shows a break in the exine which is quite characteristic of many *Quercus* grains found in peat and coal. The break is always associated with one or more of the furrows and may be more frequent in certain species than in others. The warty exine of this species resembles that of the modern species *Q. laurifolia,* and the size range is approximately the same.

Quercus leaves of twelve or thirteen species have been noted from the Fort Union series by Knowlton (1919).

Typhaceae

TYPHA **latifolipites** sp. nov. (fig. 17).

Grains irregularly spheroidal; arranged in square, rhomboidal, or irregular tetrads; shape of individual determined by tetrad arrangements; tetrad diameter 45 microns; diameter of single grains about 22 microns; germ pore in cells single, shape irregular, distribution frequently distal; exine thin, finely reticulate, reticulum continuous over tetrad, ending irregularly at pores. Holotype: Slide 351C-1.

Grains of *Typha* are fairly numerous and well preserved in the Kolarich coal. They are in all respects identical with those of the pollen of *Typha latifolia. Typha* leaves have been reported from the Paskapoo (Fort Union) rocks by Penhallow (1902).

Sparganiaceae

SPARGANIUM **globipites** sp. nov. (fig. 18).

Grains spherical; single germ pore, circular to irregular, 7–8 microns in diameter; exine 1–1.5 microns thick, delicately and irregularly reticulate; diameter 28–30 microns. Holotype: Slide 367C-5.

Sparganium pollen occurs only occasionally in the Kolarich coal. It resembles the modern species *S. eurycarpum* in exine structure, but the fossil is slightly larger in size. Fossil pollen of *Sparganium* has been observed by Thiergart (1937) in the brown coals of Germany. *Sparganium stygium* Heer is recorded by Knowlton (1919) as having been found in the Fort Union series of Yellowstone National Park.

DISCUSSION.—The Fort Union series florule from the Kolarich Coal Mine near Red Lodge, Montana, contains 17 recognized species. These represent approximately 65 per cent of the total number of species of spores and pollen found in the coal. The florule is a small one, but definitely modern in aspect. There is a striking similarity between it and certain elements of the Green River flora described from Colorado by Wodehouse (1933). This is shown by the fact that six of the recognized species of the Kolarich coal have already been described by Wodehouse. Similarity with the Green River coals of central Wyoming near the town of Wamsutter is also apparent. However, further examination of the two floras indicates that the Green River flora is much richer in species than the Kolarich coal. Some of the forms which are present in the former flora are conspicuous by their absence from the Fort Union deposit. Many varieties of fungal and fern spores, *Tilia, Engelhardtia, Platycarya, Arecipites,* and a host of polar-lengthened tricolpate pollen species were not found in the

Kolarich coal but occur in the Green River microfossil flora. The polar-lengthened tricolpate forms consist of such genera as *Rhoipites, Ailanthipites, Caprifoliipites,* and several such pollen grain genera which have not yet been described.

Miner (1935) observed only one species of spore in the Fort Union series. This, a megaspore, is described as *Selaginellites mirabilis.* The specimens were recovered from coal collected at the Homer Coal Mine, Bear Creek, Montana. This mine was exploiting the No. 4 seam. Two sections of the No. 4 seam from other mines have been examined by the authors, but no specimens of *S. mirabilis* have been found. These latter sections came from the Obertoni and Original Bear Creek Mines, both in the vicinity of the Homer Mine. Though the above fossil has not been observed, many others occur. These consist of *Laevigato-sporites* sp., *Cycadopites, Alnus speciipites, Betula* (?) sp., *Carya viridi-fluminipites, C. veripites, Momipites coryloides,* and several undescribed genera. Fossils were found to be abundant in the Obertoni Mine, but less frequent in the coal of the Original Bear Creek Mine. Though the Kolarich coal and the No. 4 coal seam deposits have some species in common, there are many forms that are different. Such dissimilarity may be considered evidence for difference in stratigraphic position. Stratigraphically the No. 4 coal lies beneath the Kolarich coal since the latter is part of either the No. 1 or No. 3 seam.

The morphological forms of pollen which occur in the Fort Union series are of some interest and may prove to have evolutionary and stratigraphic significance as other coals are studied. An examination of the pollen fossils shows that the majority are pore-bearing types. These possess one, three, four, five or six pores. The gymnosperm pollen are single-furrowed except in the case of *Sequoia,* which has a single germ pore. Two of the Angiosperm species are furrowed. One is *Quercus,* a tricolpate form, and the other is represented by a single specimen of a polar-lengthened, tricolpate species which bears a pore in the equatorial position of each furrow. This fossil is being reserved until more specimens are available for comparison. In the No. 4 seam a somewhat similar form occurs rather frequently. The coals of the Green River formation contain numerous species of this type of pollen.

A count of one thousand fossil grains was made in order to learn the relative abundance of various types of spores and pollen for possible comparison with other coal samples. All described fossils except fungi were counted. The latter were not recorded since they may represent only those types which occur on decaying vegetation. The spores of ferns and pollen of seed plants give a better conception of the Paleocene forests. Also at present, such plants appear to have greater correlative value. Table 1 gives the percentages from the count of one thousand fossils.

A study of the table will reveal that several of the described fossils do not appear in the first one thousand specimens counted. These are *Betula, Quercus,* and *Sparganium.* Two explanations can be given for their absence; first, they are themselves scarce in the Kolarich coal, and second, the great abundance of *Laevigato-sporites* and *Momipites* fossils mask all other species.

TABLE 1. *Relative abundance of microfossils in the Kolarich coal.*

Species	Per cent
Laevigato-sporites ovatus and *L. gracilis*	51.5
Momipites coryloides	32.2
Taxodium hiatipites	5.6
Carya viridi-fluminipites	4.5
Cycadopites follicularis	2.5
Carya veripites	1.8
Picea grandivescipites	.9
Alnus speciipites	.3
Typha latifolipites	.3
Sphagnum antiquaspórites	.1
Deltoidospora diaphana	.1
Sequoia lapillipites	.1
Pinus sp.	.1

The great abundance of *Laevigato-sporites* spores may be an over-representation due to the proximity of the organic substratum on which the spores fell, or the ferns may actually have had great numerical superiority to other plants. Whatever the explanation may be, fern spores are the most abundant fossils in every Mesozoic and Cenzoic coal thus far examined by the authors.

In the table will be noted 0.1 per cent of *Pinus.* This represents a poorly preserved specimen that was observed in the process of making the statistical analysis. Only one other badly corroded grain was noted, and the condition of the two grains does not at present warrant further taxonomic notice of *Pinus.*

A comparison of the Kolarich coal florule and the checklist of Fort Union plant fossils published by Knowlton (1919) reveals an interesting correlation. Eight or possibly nine of the spore and pollen genera have previously noted leaf fossil representatives. These are *Alnus, Betula, Corylus (Momipites?), Carya (Hicoria), Pinus, Quercus, Sequoia, Sparganium,* and *Taxodium.* Knowlton reports 93 genera in the total flora and numerous species in the genera *Aralia, Celastrus, Ficus, Laurus, Platanus, Populus, Sapindus, Ulmus,* and *Viburnum.* None of the latter group is as yet represented by pollen. As other Fort Union coals are studied, these genera should be found, with the possible exception of *Populus.* The pollen of this genus is thought by most micropaleontologists to be too fragile to be preserved in peat. Consequently, it cannot be expected to survive the coalification processes. *Cycadopites, Momipites (Corylus?), Picea, Sphagnum,* and *Typha* are reported for the first time in the Fort Union series. The fern spores *Deltoidospora*

and *Laevigato-sporites* are new names in the series, but the fossils may be the spores of ferns already described from leaves.

SUMMARY

Seventeen species of fossil spores and pollen are described from the Kolarich Coal Mine in the Fort Union series (Paleocene) located near Red Lodge, Montana. Ten of these species are described as new.

The florule consists of spores of fungi, *Sphagnum* moss, and ferns, pollen of the genera *Cycadopites, Sequoia, Picea, Pinus, Taxodium, Momipites, Carya, Quercus, Betula, Alnus, Typha,* and *Sparganium.*

Six of the species found in the Kolarich coal were originally described from the Green River formation (Eocene) of Colorado, and seven others have been observed in Wyoming deposits of Eocene age. However, the former pollen florule is distinct from known Eocene florules by its relatively few species with polar-lengthened tricolpate pollen grains.

A statistical analysis of the plant microfossils reveals that 51.5 per cent are ferns, 32.2 per cent *Momipites,* 6.3 per cent *Carya,* 5.6 per cent *Taxodium,* 2.5 per cent *Cycadopites,* and 1.9 per cent other recognized species.

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EFFECT OF OSMOTIC PRESSURE ON WATER ABSORPTION AND GERMINATION OF ALFALFA SEEDS [1]

Rachel Uhvits[2]

MOST STUDIES on the effect of salt concentration and specific ions on growth have dealt with plants subsequent to the early seedling stage. Since in actual agricultural practice a crop may be subjected to saline conditions from the time of planting, it is important to know the effect of salt concentration and of various ions upon the germination and growth of young seedlings. It is possible that in areas of moderate or high soil salinity, the influence of salts may be just as great or greater upon germination and early growth than it is on the later development of plants.

Alfalfa is one of the major crops of the western United States, being widely grown in areas where the salinity problem is important. Though there is a voluminous literature on germination, little is known about the influence of varying concentrations of salts or other substances upon absorption of water, germination and seedling growth of alfalfa.

In regard to the influence of osmotic pressure, Buffum (1896 and 1899) and others (Slosson and Buffum, 1898; Slosson, 1899; Stewart, 1898; Mulwani and Pollard, 1939) have reported retardation of germination and some injury to seedlings of vari-

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TABLE 1. *Per cent germination and absorption of water on NaCl substrates.*

Substrate and osmotic pressure	1 day		2 days		4 days		6 days		8 days		10 days	
	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]
Tap H_2O 0.1 atm.	36	1.48	82	3.01	86	7.58	86	9.20	88	9.40	87	10.20
NaCl 1 atm.	38	1.49	82	3.41	86	7.61	87	8.94	89	9.56	89	9.84
NaCl 3 atm.	22	1.43	73	2.12	84	6.32	84	7.78	87	8.90	90	9.63
NaCl 5 atm.	6	1.37	62	1.83	72	4.24	79	6.62	83	7.95	86	8.43
NaCl 7 atm.	0	1.31	27	1.48	57	2.93	66	5.29	83	6.22	84	7.38
NaCl 9 atm.	0	1.30	8	1.36	20	1.55	51	2.02	67	2.63	63	3.55
NaCl 12 atm.	0	1.25	0	1.34	0.7	1.34	8	1.34	15	1.41	14	1.40
NaCl 15 atm.	0	1.29	0	1.35	0	1.51	0	1.50	0	1.48	2	1.44

[a] Absorption indicated as grams of solution absorbed per gram of dry seed.

ous crops as the concentration of salt is increased. It has been demonstrated that the germination of crops may be retarded, reduced or even inhibited by large applications of mineral fertilizers (Hicks, 1900; Coe, 1923).

Some attempts have been made to determine the tolerance of seeds on substrates to which NaCl was added (Guthrie and Helms, 1903 and 1916; Harris, 1915; Harris and Pittman, 1919; Guscin, 1940). It was found that the limits of tolerance of various crop plants to salt with respect to germination varies not only with the kind of soil and amount of organic matter present (Harris, 1915; Harris and Pittman, 1919; Kearney and Scofield, 1936), but also with the species (Harter, 1905; Kearney and Harter, 1907) and even with the varieties of plants tested (Harter, 1905). Stewart (1898) reported that the germination of alfalfa seeds in sand is depressed by 0.2 per cent to 0.3 per cent and inhibited by 0.5 per cent NaCl. However, the moisture conditions or the precise concentration of the sodium chloride to which the germinating seeds were exposed were not given. It was also observed that the young seedlings of alfalfa and other crops are rather sensitive to NaCl (Kearney and Cameron, 1902; Kearney and Harter, 1907; Guscin, 1940).

The retarding effect of increased osmotic pressures on absorption of water by seeds was observed by several authors. Slosson (1899) and Slosson and Buffum (1898) found that, with most solutes employed, increased osmotic pressure resulted in decreased swelling of submerged seeds. His method, however, did not permit normal germination or seedling growth. Rudolfs (1921 and 1925) found that at 15 hours, the normally high absorption rates of alfalfa seeds are progressively retarded with increasing concentration of salt, except in very dilute solutions. Shive (1916), employing several salts in sand cultures, reported that the germination and water intake of corn and bean seeds were retarded by high levels of salt concentration. He did not use NaCl in his experiments.

GERMINATION AND ABSORPTION STUDIES IN PETRI DISHES.—*Materials and methods.*—Lots of 50 alfalfa seeds, Arizona Chilean, were weighed after elimination of small, wrinkled or imperfect seeds. These were placed in covered Petri dishes upon three layers of 9 cm. filter paper (S. & S. No. 597) with a fourth layer covering the seeds, and were moistened with 5 ml. of solution. Uniform conditions were secured by placing the Petri dishes in moist chambers in a constant temperature room at 71°F. ± 2°F. for germination periods of 1, 2, 4, 6, 8 and 10 days. Loss of water by evaporation from the Petri dishes was found to be negligible.

Sodium chloride and mannitol were used in concentrations of 1, 3, 5, 7, 9, 12 and 15 atmospheres osmotic pressure, while tap water was applied to the controls. Sodium chloride is a representative electrolyte and an important constituent of saline soils and mannitol is considered an inert non-electrolyte (Collander and Bärlund, 1933). The concentrations of these solutions were checked by freezing point determination methods and variations in concentration were kept under 5 per cent.

At the end of each experiment, the seeds or seedlings were removed from the Petri dishes with bone-tipped forceps, blotted between filter papers and weighed in tared stoppered weighing bottles. Records were made of the germination, the amount of solution absorbed, the number of deformed seedlings and hard seeds. The per cent of hard seeds was so small (about 1 per cent) that it was not considered necessary to make corrections for them in the germination and absorption studies.

Since most methods of seed sterilization affect the seed coat and therefore influence the rate of water absorption, the seeds were not sterilized. However, even in the presence of mannitol, there was little molding or decomposition except when the period exceeded ten days.

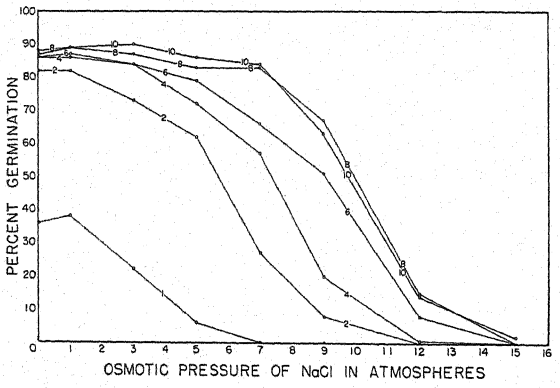
The chloride was determined by a method developed by Gauch which is a modification of existing micro-methods (Schales and Schales, 1941). The seeds and seedlings treated for four days, as described above, were dried and ground after the amount of solution absorbed was determined. Samples of 50 and 100 mg. were found to be adequate.

Experimental results.—Data on the germination of alfalfa seeds at different concentrations of NaCl and mannitol are given in tables 1 and 2. These tables and the graphs (fig. 1, 2) show that with

TABLE 2. *Per cent germination and absorption of water on mannitol substrates.*

Substrate and osmotic pressure	1 day		2 days		4 days		6 days		8 days		10 days	
	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]	Germ. %	Absor. gm.[a]
Tap H_2O 0.1 atm.	58	1.54	84	3.43	84	7.16	90	9.27	88	9.34	88	9.84
Mann. 1 atm.	59	1.59	80	3.10	86	5.77	85	6.99	88	7.91	87	7.62
Mann. 3 atm.	46	1.39	80	2.35	88	4.82	86	5.96	89	6.78	83	6.68
Mann. 5 atm.	26	1.30	74	1.95	87	3.99	86	5.02	87	5.73	84	6.04
Mann. 7 atm.	4	1.18	58	1.57	81	2.59	82	3.61	85	4.54	86	4.90
Mann. 9 atm.	0	1.13	30	1.36	76	2.37	66	2.62	83	3.88	83	3.78
Mann. 12 atm.	0	1.07	3	1.23	31	1.52	36	1.62	60	1.99	71	2.82
Mann. 15 atm.	0	1.05	0	1.13	2	1.27	6	1.34	24	1.44	57	1.53

[a] Absorption indicated as grams of solution absorbed per gram of dry seed.



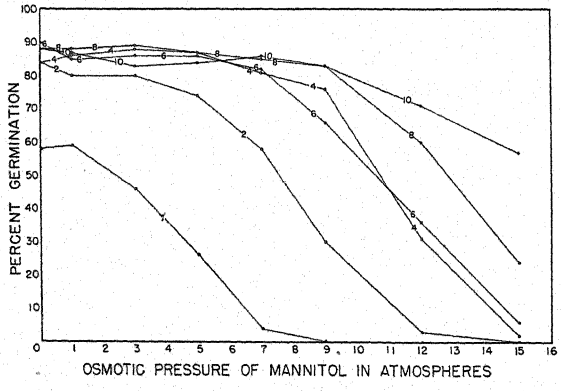


Fig. 1–2.—Fig. 1 (upper). Germination of alfalfa seeds on NaCl substrates. Numerals on the graph represent the duration of the experiment in days.—Fig. 2 (lower). Germination of alfalfa seeds on mannitol substrates. Numerals on the graph represent the duration of the experiment in days.

both NaCl and mannitol, the higher the osmotic concentration of the solution used, the lower the per cent germination, except that there was no significant difference between results obtained with tap water and solutions of one atmosphere osmotic pressure. At equal osmotic pressures the reduction in per cent germination was greater in NaCl than in mannitol. This difference was especially pronounced at the higher osmotic levels. At the same time the rate of germination was progressively depressed by higher concentrations of both NaCl and mannitol. Germination is practically inhibited on a substrate of NaCl of 12 to 15 atmospheres of osmotic pressure. At a concentration of 15 atmospheres no seeds germinated within ten days and only 2 per cent germinated thereafter.

Absorption is expressed as the increase in weight of a gram of the original seed since this increase is due to hydration of the seed and later to the intake of water by the seedling. The results of the hydration experiments (table 1 and 2 and fig. 3 and 4) show that absorption decreases as the osmotic pressure of the substrate is increased. It will be noted that where the per cent germination is very low (0 to 10 per cent) the average absorption is also very low and fairly uniform, especially on the NaCl substrates. This is true regardless of the osmotic pressure or the time interval involved. The amount of water absorbed (usually less than 1.5 grams of water per gram of seed) corresponds approximately to that absorbed during the first 24 hours of germination on a water substrate. It seems probable that this initial entry of water is imbibitional and not conditioned by the activity of the living tissues of the seed. In order to investigate this possibility, an experiment with dead seeds was carried out to determine the amount of solution that could be absorbed imbibitionally by non-living tissues of the seed.

Experiment with dead seeds.—In these experiments the seeds were killed with the heat of an infra-red lamp at 130°C. for 30 minutes or by heating in an oven at 130°C. for one hour. Germination tests indicated a complete kill with both methods. The dead seeds were normal in appearance but were lighter in weight than the viable seeds. They were treated exactly as the living seeds and were tested at all concentrations of NaCl and mannitol for 2, 4, and 6 days. These seeds swelled and often split, but none germinated.

The data in table 3 indicate that the absorption values were remarkably uniform for all concentrations of NaCl and for the three test periods. Though absorption by dead seeds on the mannitol substrates varied more than on NaCl substrates (table 3), still

TABLE 3. *Absorption of dead alfalfa seeds on NaCl and mannitol substrates.*[a]

Osmotic pressure of substrate	2 days		4 days		6 days	
	NaCl	Mannitol	NaCl	Mannitol	NaCl	Mannitol
Tap water	1.57	1.59	1.42	1.46	1.51	1.40
1 atmosphere	1.56	1.46	1.42	1.83	1.26	1.80
3 atmospheres	1.57	1.51	1.41	1.87	1.30	1.71
5 atmospheres	1.53	1.57	1.41	1.81	1.48	1.83
7 atmospheres	1.50	1.54	1.37	1.76	1.42	2.65
9 atmospheres	1.56	1.59	1.38	1.77	1.53	2.02
12 atmospheres	1.52	1.58	1.40	1.80	1.47	1.93
15 atmospheres	1.50	1.62	1.36	1.71	1.48	2.14

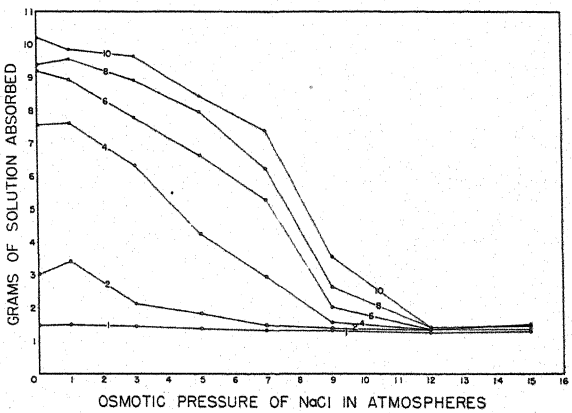
[a] Absorption indicated as grams of solution absorbed per gram of dry seed after heating at 130°C. for one half or one hour.

it remained relatively constant except in the six-day trial where difficulty was encountered in blotting the seeds as they were too soft to be handled properly. Apparently, the dead seeds reached a maximum absorption in the shortest period used, i.e., two days. Since the seeds were dead, it is assumed that imbibition was largely responsible for this absorption.

The greater absorption of normal seeds (table 1 and 2) involves osmotic forces in addition to those of imbibition. Imbibition may be an important factor in the first stages of seed absorption. It is probably the most important factor in the absorption of seeds from NaCl solutions of 15 atmospheres of osmotic pressure, regardless of time interval. This conclusion is supported by the results of the recovery studies reported below. A large proportion of the seeds in contact with NaCl at 15 atmospheres of osmotic pressure were dead (table 4) and their absorption was probably due to imbibition. With mannitol at 15 atmospheres of osmotic pressure, though many seeds remained alive (table 4), their absorption rate was so slow that the amount of liquid absorbed was less than that of the dead seeds in all but the longest periods involved. In these longer periods in mannitol, germination had actually begun, and the osmotic forces were in operation (table 2).

Experiments on recovery of seeds from the effect of high salt concentration.—It has been noted that viable seeds on substrates of 12 and 15 atmospheres osmotic pressure germinated very poorly and that this was more pronounced with NaCl than with mannitol. In order to determine whether or not the non-germinated seeds were dead or would recover under more favorable conditions for germination, they were transferred to clean Petri dishes on filter paper moistened with water. They were then placed in moist chambers at constant temperature for seven days. The germination under these conditions was determined and the per cent recovery calculated. Recovery was taken to be the per cent of germination of seeds after transfer to the water substrate. With one exception the results in table 4 indicate that the recovery was greater with mannitol than the corresponding NaCl solutions. This difference is particularly striking on the 15-atmosphere substrate where the recovery was much greater and the per cent of deformed seedlings much smaller than in NaCl.

Entry of chloride.—The greater recovery of seeds germinated in mannitol solutions of high osmotic



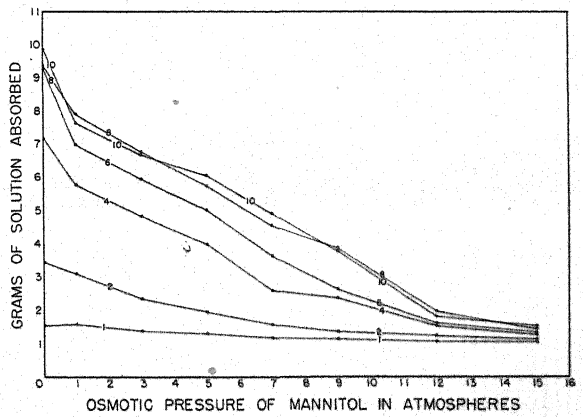


Fig. 3-4.—Fig. 3 (upper). Absorption of NaCl solutions by alfalfa seeds. Numerals on the graph represent the duration of the experiment in days.—Fig. 4 (lower). Absorption of mannitol solution by alfalfa seeds. Numerals on the graph represent the duration of the experiment in days.

TABLE 4. *Recovery of treated seeds.*

Duration of initial treatment days	Substrate	Osmotic pressure of initial treatment: 12 atmospheres — Germ. on initial substrate %	Seeds not germ. %	Recovery on H_2O substrate %	Deformed seeds %	15 atmospheres — Germ. on initial substrate %	Seeds not germ. %	Recovery on H_2O substrate %	Deformed seeds %
6	NaCl	3	97	63	7	0	100	23	10
	Mannitol	37	63	83	1	6	94	85	3
8	NaCl	28	72	75	16	0	100	22	12
	Mannitol	61	39	62	1	20	80	79	7
10	NaCl	14	86	56	14	2	98	22	16
	Mannitol	70	30	67	2	34	66	79	1

pressure as compared with those of equal osmotic pressure on NaCl substrates, and the larger number of abnormal seedlings under NaCl treatment suggested that the entry of toxic ions might be a factor in this differential response. The four-day germination period was selected and the accumulation of Cl in the seeds was determined for untreated seeds, those in tap water, and those on all NaCl substrates. The data on accumulation are given in table 5. These show that on a dry weight basis chloride increases with increasing osmotic pressure, reaching a peak at 7 atmospheres with another high point at 15 atmospheres. A few Cl analyses of dead seeds subjected to varying concentrations of NaCl for four days were made for purposes of comparison (table 5). At 15 atmospheres, the dead seeds contained even more chloride than did the normal seeds.

While the amounts of Cl on a dry weight basis decreased in the 9 and 12 atmosphere range, the accumulation of Cl in relation to the water absorbed increased with the increasing concentration of the substrate and exceeded 1 per cent under the 15-atmosphere treatment. This may account for the low per cent germination of seeds in high concentrations of NaCl and the lower recovery of seeds subjected to such treatments.

GERMINATION STUDIES IN SAND CULTURE.—*Materials and methods.*—Two experiments of similar design were set up with 12 ten-gallon crocks filled with sand and automatically irrigated as described by Gauch and Wadleigh (1943). Seven treatments were used: a control with tap water, and six salt treatments in which NaCl was added to produce osmotic pressures of 1, 3, 5, 7, 9 and 12 atmospheres. There were two replicates within each experiment except for the control and 12-atmosphere treatments. The concentrations of the solutions were checked at the beginning and end of each experiment by conductivity methods and the pH of the solutions was maintained in the range of 6.0 to 6.5 with additions of sulfuric acid.

In each pot 100 alfalfa seeds, Arizona Chilean, were planted one half inch deep and records were kept on the emergence of seedlings above the surface of the sand.

Experimental results.—The final total germination for the two experiments based on emergence above sand is given in table 6.

In general, germination in sand followed the same pattern as germination in Petri dishes. There was little difference in the total germination of seeds supplied with tap water and those receiving the one-atmosphere NaCl solution. In all other cases, the higher the osmotic pressure, the lower the per cent germination and the slower the emergence of seedlings. As compared with the control, the retardation was 1, 2, 4, and 6 days for the NaCl treatments of 3, 5, 7, and 9 atmospheres. In experiment II, the germination values were higher than in experiment I, but the seedling mortality under the high salt treatments was greater. Although seven seedlings emerged at the 12-atmosphere level in experiment II, all of them died without producing trifoliate leaves, and very few seedlings at seven

TABLE 5. *Accumulation of chloride in alfalfa seeds after 4 days of treatment.*

Treatment	m.e. Cl per 100 g. dry wt.	% Cl dry wt. basis	gm. soln. absorbed per gram seed	gm. Cl per 100 gm. of H_2O absorbed	Dead seeds % Cl dry wt. basis
Dry seeds not treated.	1.29	0.046	0.0	0.0	...
Tap H_2O	1.25	0.044	7.58	0.059	...
1 atm. NaCl.....	13.8	0.49	7.61	0.064	0.42
3 atm. NaCl.....	33.3	1.183	6.32	0.187	...
5 atm. NaCl.....	43.2	1.535	4.24	0.362	...
7 atm. NaCl.....	48.3	1.719	2.93	0.586	...
9 atm. NaCl.....	34.0	1.208	1.55	0.776	1.34
12 atm. NaCl.....	34.7	1.233	1.34	0.920	...
15 atm. NaCl.....	50.4	1.79	1.51	1.091	2.15

TABLE 6. *Emergence of alfalfa seedlings in sand cultures.*

Exp. No.	Per cent germination: Tap H_2O	1 atm. NaCl	3 atm. NaCl	5 atm. NaCl	7 atm. NaCl	9 atm. NaCl	12 atm. NaCl	Greenhouse temperatures[a] Maximum	Mean
I	84	73	72	42	23	3	0	99°F.	79°F.
II	85	84	91	79	79	44	7	92°F.	74°F.

[a] The greenhouse maximum and mean temperatures of the first week after planting.

and nine atmospheres osmotic pressure lived long enough to produce true leaves.

The lower germination in experiment I may have been due to higher temperatures during the germination period of that experiment (table 6). Clear weather during experiment I resulted in higher day temperatures at the surface of the sand while foggy weather during the first week of experiment II resulted in lower surface temperatures. When bright sunshine returned during the second week of experiment II, many of the emerged seedlings died before their cotyledons were fully open. Many more died at a more advanced stage of development, especially in the higher salt concentrations.

The seedlings in experiment I were allowed to grow for one month and at the end of that period they presented a consistent and clearly graded series in regard to the size of tops and length of roots (fig. 5).

EFFECT OF OSMOTIC PRESSURE ON SEEDLING ROOTS.—One hundred alfalfa seeds were placed in large enameled pans between four layers of sterile cotton flannel, one layer covering the seeds. The flannels were moistened with solutions of NaCl adjusted to 1, 3, 5, 7, 9, and 12 atmospheres osmotic pressure. Distilled water and tap water were used as controls. The pans were covered and placed in a germinator at room temperature, which fluctuated between 66°F. and 82°F., with mean temperatures of 76°, 73° and 75°F. for the three experiments in this series. Daily records of germination were kept and at the end of six days, the roots of 20 healthy seedlings selected at random in each culture were measured.

Data for the per cent germination of 300 seeds and the average length of an equal number of seedling roots under each treatment are given in table 7. These indicate a steady decrease in the length of the seedling roots as the concentration of salt increased. The germination values are in line with those reported above (table 1).

DISCUSSION.—The results obtained indicate that an increase in concentration of the substrate decreases the rate of entry of water into the seed and that this in turn retards germination.

There are distinct differences in the action of NaCl and of mannitol at equal osmotic pressures. Mannitol is a soluble non-electrolyte which probably enters plant cells slowly if at all (Collander and Bärlund, 1933). The drop in absorption with increased concentration of mannitol is much more gradual than with NaCl (fig. 3, 4). At high concentrations, the total absorption values with mannitol are greater than with NaCl; consequently, if given enough time, relatively high germination rates are obtained with mannitol solutions of 12 and 15 atmospheres (fig. 2). Thus, the effect of increasing the osmotic pressure of mannitol solutions seems to be largely osmotic in reducing the entry of water

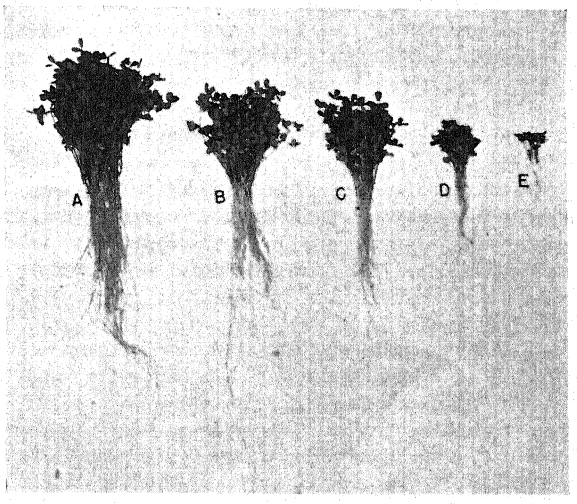


Fig. 5. Alfalfa seedlings grown in sand cultures (exp. I). (A) Tap water. All others are on NaCl substrates with osmotic pressures as follows: (B) 1 atm.; (C) 3 atm.; (D) 5 atm.; (E) 7 atm.

TABLE 7. *Germination and root length of alfalfa seedlings.*[a]

	Distilled water	Tap water	NaCl — osmotic pressure in atmospheres 1	3	5	7	9	12
Per cent germination	87	87	86	83	76	47	26	3
Root length, cm.	5.4	5.6	5.2	4.3	3.3	2.1	1.4	..

[a] Averages of three replicates of 100 seeds per treatment.

into the seeds and seedlings. At equal osmotic pressures, solutions of sodium chloride cut down water intake even more than does mannitol suggesting the operation of some additional factor (fig. 3).

That NaCl at the higher concentrations is toxic to germinating alfalfa seeds is supported by the recovery studies reported in table 4. The recovery of seeds transferred from NaCl at 12 atmospheres to tap water is considerably greater than the recovery of seeds treated with NaCl at 15 atmospheres osmotic pressure for the same period of time. This does not apply to the treatment of seeds with mannitol, as in this case the recovery is very nearly the same for both the 12- and 15-atmosphere treatments (table 4). This indicates a greater seed mortality at 15 atmospheres than at 12 atmospheres osmotic pressure of NaCl. Table 4 also shows a greater proportion of deformed seedlings at 15 atmospheres than at 12 atmospheres of NaCl. The number of deformed seedlings is much greater in all concentrations of NaCl than in the mannitol solution. All these facts indicate that NaCl has a detrimental effect upon alfalfa seeds and seedlings in addition to its osmotic effect.

Chloride analyses indicate that increasing amounts of Cl were absorbed by the seeds and seedlings of alfalfa as the osmotic pressure of the substrate was increased (table 5). The Cl values at 9 and 12 atmospheres osmotic pressure correlated with low water intake and low germination at these concentrations, while the chloride content of seedlings at 5 to 7 atmospheres of osmotic pressure may be explained on the basis of the larger absorbing surface of seedling roots and greater entry of water (table 7). The relatively high Cl value at 15 atmospheres may have been due to a large proportion of dead seeds allowing free diffusion of NaCl (table 5). When the chloride accumulation in alfalfa seeds and seedlings treated with NaCl is considered on the basis of amount of water absorbed, a gradual upward trend is noted (table 5).

The greenhouse experiments in sand cultures confirm the results obtained in the germination studies in Petri dishes. They also illustrate the great sensitivity of young seedlings to relatively high concentrations of NaCl which accounts for the high seedling mortality in cultures at 7 and 9 atmospheres osmotic pressure. There is evidence that increased temperature increases the seedling mortality at high concentrations of NaCl (Ahi and Powers, 1938; Ogasa, 1939).

SUMMARY

The germination of alfalfa seeds was tested on substrates supplied with solutions of NaCl and mannitol at osmotic pressures ranging from 1 to 15 atmospheres. These tests were carried out in Petri dishes and sand culture.

In all experiments, the higher the concentrations of NaCl or mannitol the lower were the rate and the per cent germination of alfalfa seeds.

The retardation and reduction of germination were greater on NaCl than on mannitol substrates at equal osmotic pressures.

Germination of alfalfa is practically inhibited on NaCl solutions of 12 to 15 atmospheres.

The hydration of seeds decreases as the concentration of the substrate increases with either NaCl or mannitol. Where the per cent germination is very low, the average absorption is also uniformly low.

Differences in response to the two substrates at isosmotic concentrations suggests a toxic effect of NaCl. This effect increases with increase in concentration of NaCl.

The absorption of chloride increases with the increase of NaCl in the substrate.

A measure of comparative injury produced by NaCl and mannitol solutions is shown by the greater per cent recovery and the smaller number of deformed seedlings on mannitol substrates of high concentration than with isosmotic concentrations of NaCl.

The higher the concentration of NaCl in the sand culture solution, the greater was the seedling mortality.

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STUDIES OF DEVELOPMENT IN LONG SHOOTS AND SHORT SHOOTS OF GINKGO BILOBA L. I. THE ORIGIN AND PATTERN OF DEVELOPMENT OF THE CORTEX, PITH AND PROCAMBIUM [1]

James E. Gunckel and Ralph H. Wetmore

THIS INVESTIGATION on the Maidenhair Tree, *Ginkgo biloba* L., is limited to a consideration of studies carried out on the development of the shoot system. There are two well-defined types of shoots found in *Ginkgo,* namely, *long shoots* and *spur, dwarf,* or *short shoots*. In gross appearance, a long shoot is that growth habit in which the leaves are separated by regular internodal intervals all along the stem (fig. 1). By contrast, a short shoot has no appreciable internodes so that the leaves appear aggregated at the stem tip as if in a whorl (fig. 3). This dual shoot expression takes on an added interest since, in geological history, it seems clear that the short shoot habit was already established in various Ginkgophytes during the Mesozoic era.

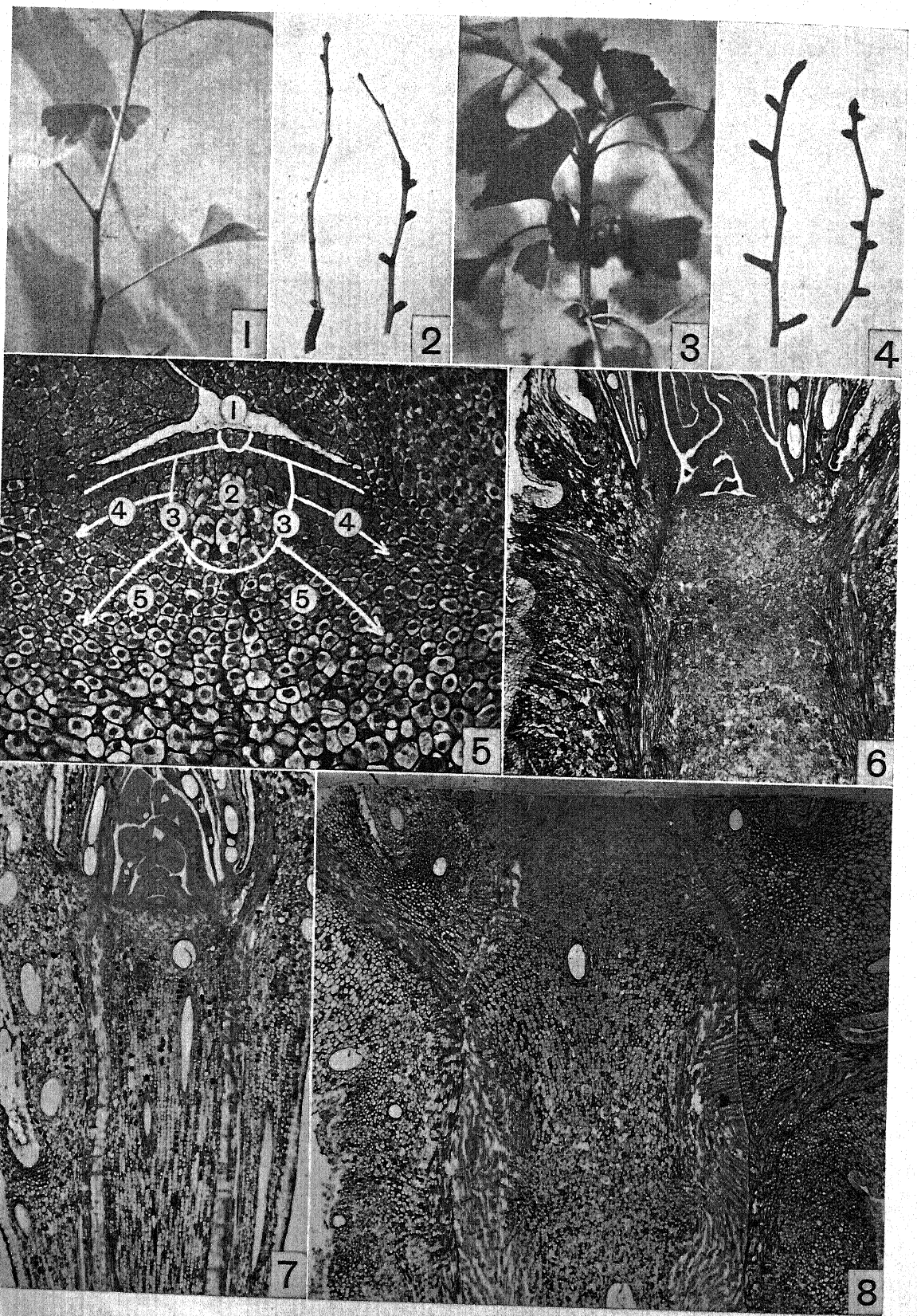
In point of time, the Ginkgoales may well have been present in the Paleozoic era. Seward (1919), however, considers that the evidence in most cases is wholly inadequate to establish this thesis. No case is known from this era of *Ginkgo*-like leaves in attachment. During the Mesozoic and Cenozoic eras, there seems to be little question that the Ginkgophytes became world-wide. Indeed, in the Cenozoic, one form, *Ginkgo adiantoides* (Unger) Heer was quite widespread and resembled the living species to such an extent that many authors consider two species unjustified (Heer, 1868; Seward, 1919). Florin (1936), however, still prefers to maintain the earlier form as a distinct species.

Florin (1936) described many Mesozoic form-genera as Ginkgophytes. Of these, *Arctobaiera, Czekanowskia* and *Phoenicopsis* were forms in which a deciduous shoot, surrounded at the base by persistent needle-like leaves, bore a varying number of fascicled foliage leaves. A similar form, *Torellia,* was described from the Tertiary. Heer (1877b) described a species of *Phoenicopsis* with a short shoot bearing twenty-one leaves.

Although leaves of *Ginkgoites* and *Baiera* are abundantly represented in fossil floras, very little is known of the habit of the foliage shoots. The type *Ginkgo digitata* (Brogn.) Heer from the Jurassic approaches very closely to *Ginkgo biloba* in the variations of its leaves but the short shoot ascribed to this form (Heer, 1877a) is doubtful since it has no leaves in attachment. Seward (1919) points out that in a few cases there is evidence of the occurrence of several leaves on a single short shoot, as in *Baiera parecipartita* Nat. Harris (1931) concludes that in *Baiera,* as in *Czekanowskia, Phoenicopsis* and *Hartzia* (Harris, 1926, 1935), the leaves are borne in groups on scaly short shoots. He suggests that the rarity of short shoots, when compared with the number of isolated leaves found in the same deposits, may indicate that the leaves were shed before the shoot, like those of the present species.

From the literature and even more from an extensive survey of individual *Ginkgo* trees over a five-year period, it has become evident that shoot expression is a dynamic problem. Thus, each spring, opening buds initially form short shoots; that is, as the buds open, the developing leaves enlarge with little or no internodal elongation. This is true for terminal as well as for lateral buds. At this time it is impossible, from their external expression, to determine

[1] Received for publication September 5, 1945.


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whether these shoots will eventually become short or long shoots. After some weeks certain of the shoots, some terminal, some lateral, give indications of internodal elongation, and long shoots have their inception. This change to long shoot habit is not sudden and simultaneous over a tree, but may occur on any shoot at any time during the growing season. It should be noted that a short shoot of many seasons may in any season assume the long shoot habit (fig. 2). Of equal significance is it that a long shoot of one or more previous seasons may in any season fail to produce internodal growth and so assume the short shoot habit (fig. 4). The developmental studies herein reported will afford a basis for physiological studies upon the variability of shoot growth in *Ginkgo*.

The description by Foster (1938) of the cellular organization of the shoot apex in *Ginkgo* is complete and accurate in detail so that a further report of this meristematic region is unnecessary. From his investigation Foster concluded, and this is confirmed in the present study, that there is no fundamental difference in the cellular organization of the apical meristems in the long and short shoots. He segregated the cells of the shoot apex into five well-defined zones (fig. 5)—the apical initial group (1), the zone of central mother cells (2), the transition zone (3), the peripheral subsurface layers (4), and the pith rib meristem (5). These zones are characterized by their position, the direction and rate of growth of their cells, the size and thickness of their cell walls and their staining reactions.

By anticlinal and periclinal divisions, the group of apical initials (1) gives rise to the surface layer and to the central mother cells (2). From the surface layer the epidermis originates, though occasionally periclinal divisions contribute to the peripheral subsurface layers (4). From the central mother cells, there is derived a transition zone (3). The divisions which occur in this zone are predominantly parallel to the surface of the mother-cell zone and give rise on the flanks to the peripheral subsurface layers (4), and at the base to the pith rib meristem (5). From the outer and inner subsurface layers arise respectively the cortex and the procambium. Further local developments in this zone initiate the formation of bud scales and foliage leaves. The pith rib meristem (5) produces a rapidly maturing pith a short way below the zone of central mother cells.

The present investigation continues Foster's study by relating to this apical meristem the origin and development of the primary plant body in both long and short shoots. The first paper considers the origin and pattern of development of the cortex, pith and procambium, with special attention given to the development of procambium in relation to leaf primordia. The second paper deals with the later progressive development of primary xylem and phloem and with the relation of the primary vascular strands to the organization of *Ginkgo* shoots.

As a working basis for this investigation, the authors have adopted the terminology and concepts of Esau (1943).

MATERIALS AND METHODS.—Materials were collected locally from staminate and ovulate trees. To allow for any seasonal variations these collections were made: (1) in May and early June when buds first opened; (2) in late June and early July during the period of shoot elongation; (3) in late July and early August when the plants were setting buds; (4) in the winter dormant period. Comparable collections were also made from young plants grown in the greenhouse.

For rapid penetration of killing and fixing agents, outer bud scales were removed and the stem tips gashed and evacuated. Formalin-acetic acid-alcohol, Navashin's, and the Allen-Wilson modification of Bouin's killing and fixing agents were used. Navashin's reagent gave the best results in sectioning apices of *Ginkgo*, always more or less difficult. The material was soaked in dilute hydrofluoric acid for several days to soften the wood and to dissolve the numerous druses of calcium oxalate. It was then dehydrated in an ethyl alcohol—normal butyl alcohol series (Zirkle, 1930) and imbedded in "Tissue Mat." Serial longitudinal and transverse sections were cut at 10 micra and stained with a combination of iron alum—hematoxylin and safranin according to the schedule of Jeffrey (1917).

In the course of these developmental studies it became necessary to establish the phyllotactic relationships of the leaves. The method adopted was as follows: using a microprojector, diagrams of serial transverse sections of the stem apex were made at 50 micra intervals. The diagrams thus made permitted the following of the young leaves from tip to attachment. The orderly sequence in the appearance of leaves thus became clear. In all ma-

Fig. 1–8.—Fig. 1. Growth habit of long shoot on 4-year old seedling. Note internodal elongation and bud in axil of each leaf. ×2/5.—Fig. 2 (left). Lateral short shoot which has assumed the long shoot growth habit; (right) long shoot which assumed the short shoot habit for several seasons and then resumed growth as a long shoot. ×1/3.—Fig. 3. Terminal short shoot of 6-year old seedling. Note short internodes and leaves aggregated at the apex. Lateral buds of long shoot have all become short shoots. ×1/3.—Fig. 4 (left). Long shoot which assumed the short shoot terminal habit; (right) the same, except that this phenomenon has occurred earlier. ×1/3.—Fig. 5. Meridian longitudinal section through the apex of a terminal short shoot of *Ginkgo* showing the following zones of growth: Zone 1 is the apical initial group from which, by anticlinal and periclinal divisions respectively, the surface layer and the central mother cells originate; Zone 2, in which volume growth predominates, is the prominent cup-shaped mass of large, slowly dividing central mother cells; Zone 3 is characterized by the small size and frequency of division of its cells. It gives rise to the peripheral subsurface layers (Zone 4) and the pith rib meristem (Zone 5). ×180.—Fig. 6. Median longitudinal section of lateral short shoot. ×19.—Fig. 7. Median longitudinal section of terminal long shoot. ×16.5.—Fig. 8. Near-median longitudinal section of terminal shoot in which the pith development reflects successively, from the base upward, a long shoot, a short shoot, a long shoot and a short shoot. ×17.

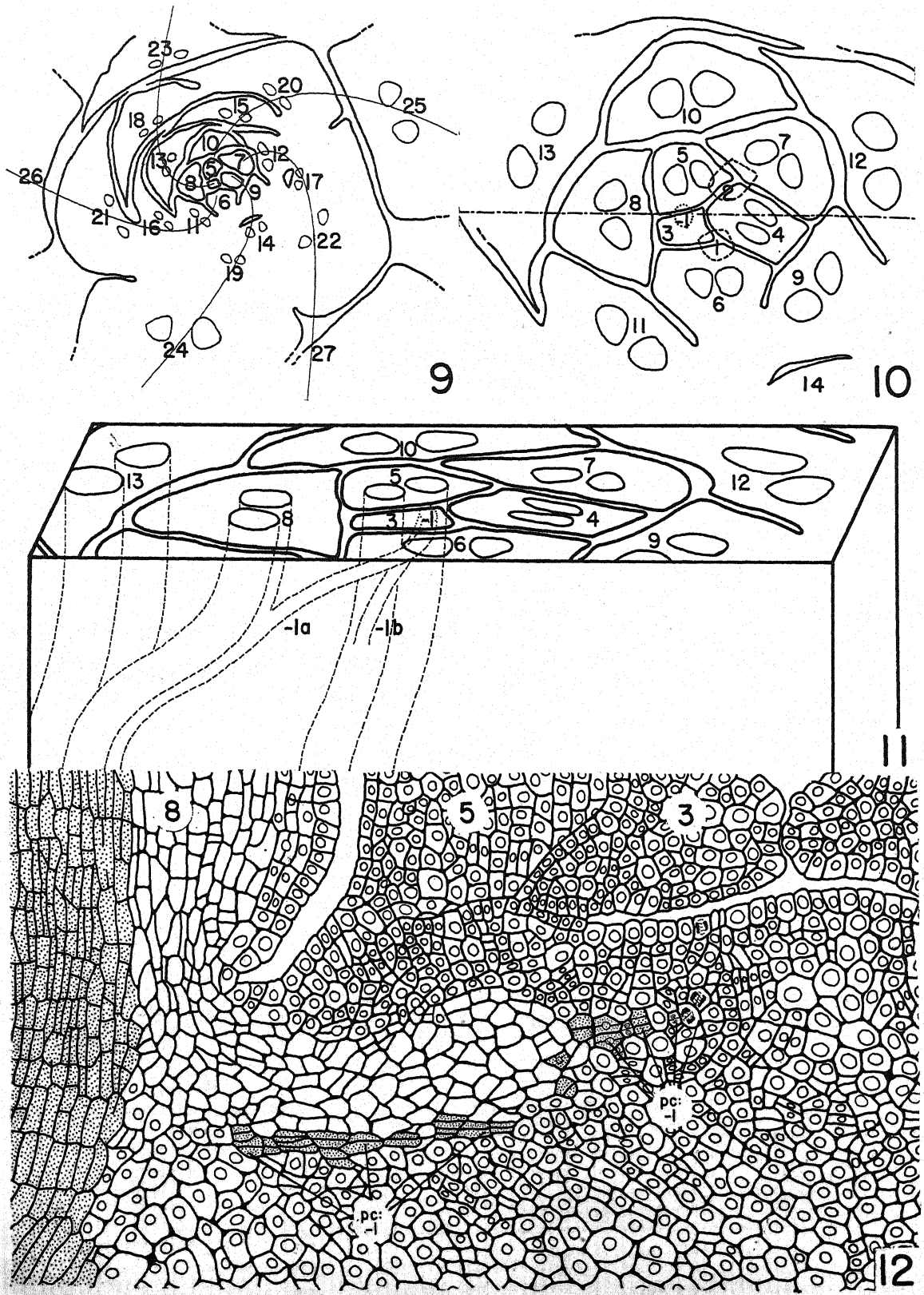


Fig. 9–12.—Fig. 9. Diagram of the transverse section from a terminal short shoot taken *just above* the shoot apex. The phyllotactic "spiral" is 5/13 or 5 + 8.—Fig. 10. The positions of the youngest leaves not shown at the level of

terial studied, from many trees, the phyllotaxis proved to be 5/13 (or 5 + 8).

Esau (1942) and Crafts (1943a) have pointed out the difficulties of describing procambial development in relation to the appearance of leaf primordia. Their experience demonstrated the value of reconstructing this development from serial longitudinal sections. To do this, two complementary techniques were used in this study. First, the phyllotaxis of a tip was determined, as previously indicated, by a series of transverse sections of a bud cut *just above* the shoot apex. Secondly, the same tip was then cut in serial longitudinal section to follow the longitudinal course of procambial development in relation to each leaf. Microprojections of these serial longitudinal sections of the stem tip, at intervals of 50 micra, were then drawn at a convenient magnification. When the drawings were cut out and bound together with inserts of the proper thickness to represent the sections omitted, they gave a reasonably accurate, enlarged, three-dimensional reconstruction of the shoot apex. These drawings were particularly useful for reconstructing the youngest leaves, which did not show at the level of the lowest transverse section before the tip was cut into longitudinal sections. The position of these reconstructed leaves was then accurately plotted in the diagrams used to establish the phyllotaxis.

ORIGIN AND DEVELOPMENT OF THE CORTEX.—As just indicated, the peripheral subsurface layers, throughout the growing season, add longitudinally to the pre-existing cortex and also give rise to new bud scales and leaves. Initially the new cortical cells are homogeneous in appearance.

The young leaf primordia arising from these peripheral layers have their free tips oriented inward over the apical meristem (fig. 6, 7). With the appearance of successive new primordia, the older primordia are gradually turned outward through an arc of 90° or more so that they appear on the outer flanks of the broad, rather flat apex (fig. 6, 7). This reorientation of leaves and the initial crowding of the leaf bases make it difficult to follow accurately the changes of the peripheral subsurface cells.

The first indication of differential change in the peripheral cells below an area of leaf initiation is in the vacuolation of the cells in a more or less definite zone. This zone has its incidence in the progressive acropetal spread of the vacuolation[2] associated with the older subjacent and flanking leaf primordia; its path (fig. 12) gradually becomes recognizable across the peripheral subsurface layers and thence along the abaxial side of each leaf primordium. When successive, abaxially vacuolated leaf primordia, and their subjacent vacuolated buttresses, have surrounded the stem apex, the vacuolated cortex characteristic of long or of short shoots has taken form at that level.

In the short shoot, the leaves remain aggregated at the stem apex because of no appreciable internodal elongation. The annual increment in length is small in such shoots. This is correlated with the absence of repeated transverse cell divisions in the cortical derivatives of the peripheral subsurface layers. These cortical derivatives quickly become mature vacuolated cortical parenchyma (fig. 6). They are characterized by a greater breadth than height, and reflect in their development irregular cell division and cell enlargement which have resulted in, or have been accompanied by, large intercellular spaces. The cortex of a short shoot is, therefore, quite broad and spongy, and retains this character since it is subject to very little crushing from the later-appearing, limited, secondary activity.

In the long shoot, the leaves are separated by conspicuous, internodal intervals all along the stem. The original increment to the cortex from the derivatives of the peripheral subsurface layers is the same in long and short shoots. In the long shoot, however, this increment is stretched in length so that it never attains so conspicuous a width as in the short shoot. Here vertical files or ribs of cells remain as evidence of prolonged, orderly, transverse divisions within the young cortical cells situated just below the shoot apex (fig. 7). These parenchyma cells are characterized by greater height than breadth, by relatively uniform cell enlargement, and, since the linear order of the cells comprising the ribs is not displaced during growth, by very small intercellular spaces. The cortex of a long shoot is, therefore, radially less thick and its cells are more compactly arranged than those of a short shoot. Furthermore, this cortex becomes sub-

[2] In this study, the terms *vacuolation, vacuolated,* etc., are used in a descriptive sense only. No implication of a more specialized nature nor of a more highly differentiated state is intended when cells are described as more highly vacuolated.

figure 9 are here plotted into the established "spiral" of 5/13. The broken line across the figure indicates the plane of section of figure 12.—Fig. 11. Three-dimensional diagram to show the path of the two strands to leaf -1, the next leaf to appear in the apex. One strand develops acropetally from the base of the next lower leaf in the same "spiral" (leaf 8, -1a), and one from an adjacent "spiral" (leaf 5, -1b).—Fig. 12. Longitudinal section of a terminal short shoot, cut in the plane of section indicated in figure 10, showing the development of one procambial strand to leaf -1. Drawing traced from a photograph; procambial cells are stippled; highly vacuolated cells are demarcated in the drawing by omission of the nuclei. The adaxial vacuolation of leaf 8 is shown continuous with a zone of highly vacuolated cells below the buttress of leaf 5. This zone of vacuolated cells can also be demonstrated to be in continuity with similarly vacuolated cells from the adaxial side of leaf 5 and from the leaf gap region of the confronting leaf (leaf 13, fig. 10, in this case). Dividing, recently divided and elongating procambial cells are noticeable adjacent to the zone of abaxial vacuolation of the presumptive leaf primordium. Successive sections establish the continuity of this strand with that of leaf 8. Part of the strand which is developing on one side of the presumptive leaf gap is shown. Active cell divisions will complete its continuous acropetal development to the predicted area of leaf initiation.

ject to crushing from the extensive secondary activity.

ORIGIN AND DEVELOPMENT OF THE PITH.—The zone of pith-producing rib meristem in the shoot apex characteristically produces chains of small, actively dividing cells. However, derivatives of these cells quickly develop into a rapidly maturing pith (fig. 5). Progressive changes in these rib cells and their derivatives may be easily followed back from the apex, since they are not influenced by secondary activity nor by departing leaf traces, as is the case in the derivatives of the peripheral layers.

In the short shoot, the rib meristem produces a few short chains of cells each season and suspends its meristematic activity (fig. 5). This is correlated with the short annual increment in length. Immediately below this level, however, some of the derivatives of this rib meristem undergo longitudinal divisions and all of the derivatives increase very appreciably in size, though still characterized by a slightly greater breadth than height. These seemingly unordered cell divisions and unequal cell enlargements displace the characteristic linear arrangement of the ribs. There is an associated development of large intercellular spaces (fig. 6). At even a short distance below the apex, the pith of a short shoot appears therefore as a broad, spongy cylinder of large, more or less isodiametric parenchyma cells, with extensive intercellular spaces.

In the long shoot, the pith-producing rib meristem retains its capacity to divide rapidly, as shown by the long chains of derivatives which remain as evidence of prolonged, orderly, transverse divisions (fig. 7). These files of derivatives, by subsequent cell enlargement, attain approximately the same transverse diameter as the derivatives of the rib meristem in the short shoot. However, mature cells in the pith of the long shoot are characterized by slightly greater height than breadth, by very small intercellular spaces, and by uniform transverse cell division, so that the linear chains of cells are not lost. Toward the end of a growing season, however, when elongation stops, this linear order is displaced by a development similar to that described for short shoots.

The usual reversion of the long shoot growth pattern to that of the short shoot at the end of each growing season, as well as the universal short shoot expression at the beginning of each season, suggests that the short shoot is the present characteristic type of *Ginkgo*, and that the long shoot is expressed only if some influence or influences, still to be determined, is active.

The zone of transition from short to long shoot or vice versa (fig. 2, 4) shows, in a median longitudinal section, a shift in the growth pattern of the pith corresponding to the change in type of shoot growth. This shift may be illustrated in a shoot that was considered a terminal short shoot on the basis of external appearance. The tip shown here (fig. 8) was collected in late July when the plants were setting buds so that the current annual increment of growth in length was already completed. Just above the lower end of the section is shown a small band of slightly broadened, spongy pith characteristic of a short shoot or even of a long shoot at the end of the growing season. Above this band there are the chains of rib derivatives characteristic of the long shoot. Finally, when elongation ceased, as is apparent just below the pith rib meristem, the typical short shoot expression appeared again. This shoot was probably intermediate between the two shoot-types. The investigation suggests that any differences between pith development in long and short shoots are essentially quantitative rather than qualitative.

No consideration of the development of the pith would be complete without mention of the vacuolating finger-like processes which develop acropetally from the pith across the peripheral subsurface layers into the adaxial sides of leaf primordia. It is probably significant from a developmental point of view that each of these processes is associated with the developing foliar procambial strands. To each leaf develops a so-called "double bundle." Of the two strands comprising this "double bundle," one passes to either side of the developing, vacuolated process in what is called the leaf gap region[3] to become parallel strands above the gap. It should be pointed out that these finger-like processes add to the vacuolation of the cortex, though usually they form at least one plastochrone later than those abaxial zones which proceed acropetally from leaf primordium to leaf primordium. The large leaf gaps, and the consequent crooked path of the traces, increase the problems of following the individual traces and, even more so, of illustrating them.

ORIGIN AND DEVELOPMENT OF THE PROCAMBIUM.—As already pointed out, the procambium arises from the inner cells of the zone of peripheral subsurface layers in the stem apex (Zone 4, fig. 5). A study was made of transverse and longitudinal sections in both long and short shoot apices in order to determine when and in what relation the procambium is initiated in this region. In the orderly plastochronic succession of young leaves, a transverse section just above the stem apex shows progressive stages of development in the successive leaf primordia. Leaves 1 through 4, photographed from such a transverse section, are shown in figures 13 to 16; the outlines of the "double bundle" in each are artificially delineated.

Most evident in leaf 1 (fig. 13) is an area of large, abaxial, highly vacuolated cells (*abv*) which, in successive transverse or in longitudinal sections, can be demonstrated to be in continuity with the similarly vacuolated cells from the leaf gap region

[3] Inasmuch as *Ginkgo biloba* possesses interfascicular parenchyma which is vacuolated as early as the finger-like processes from the pith, it becomes impossible to determine the flanking limits of the leaf gap. In consequence, any consideration of the leaf gap as such is omitted from this article.

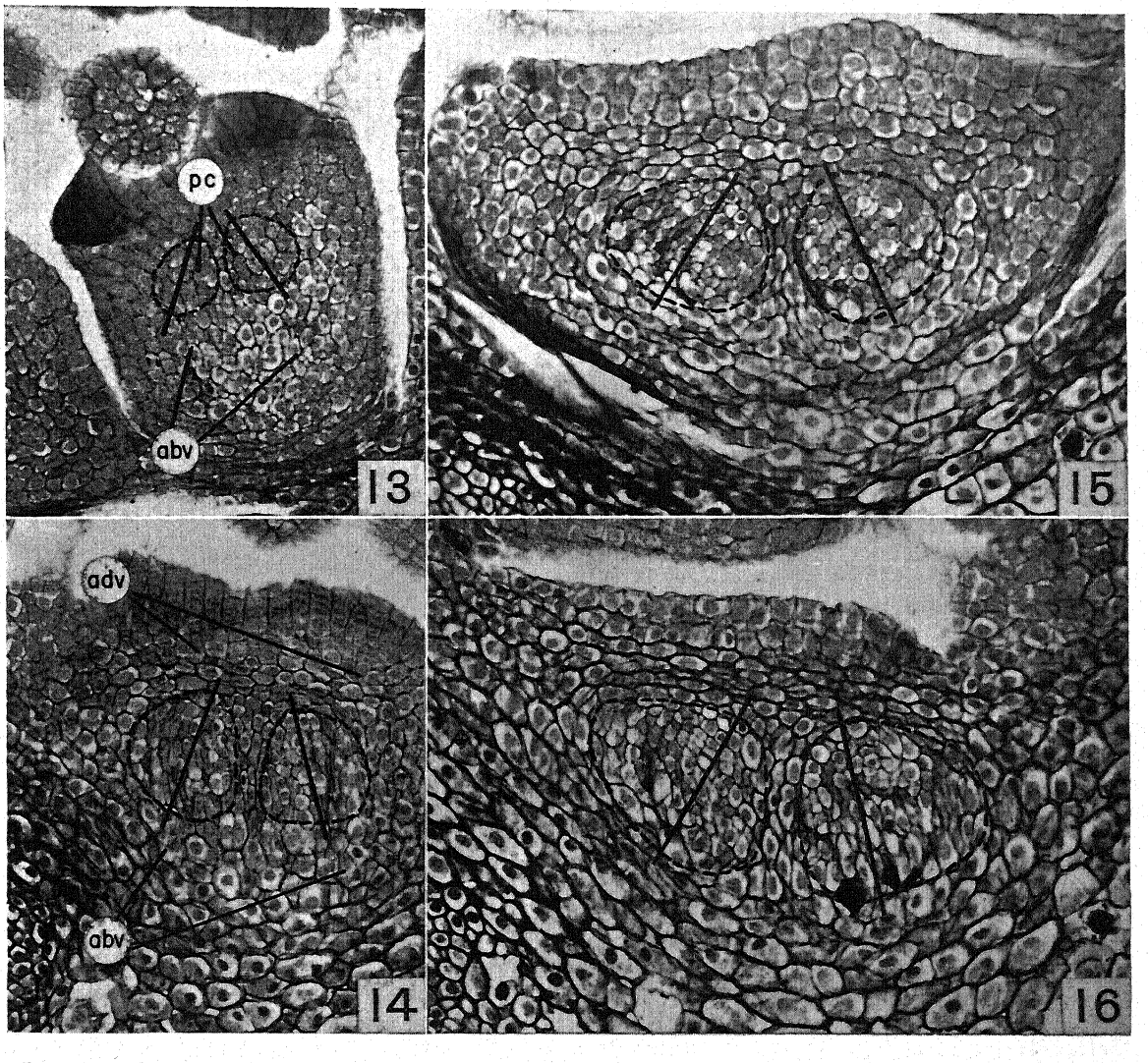


Fig. 13–16. Sections of leaves 1 to 4 from a single transverse section cut at the level of the apex of a long shoot. These leaves are oriented so that the inner side of each leaf fronting on the apical meristem is uppermost. The double leaf strand is artificially delimited.—Fig. 13. Leaf 1 showing area of large, abaxial, highly vacuolated cells (*abv*); just inside this area are the two very young procambial strands (*pc*). ×185.—Fig. 14. Leaf 2 with a larger double strand between regions of ab- (*abv*) and adaxial (*adv*) vacuolation, showing growth by scattered periclinal divisions through the body of the bundles and by anticlinal divisions on the flanks. No active divisions happen to show in this section. ×185.—Fig. 15. Leaf 3 in which *polarized* periclinal divisions in the bundles predominate. ×240.—Fig. 16. Leaf 4 showing that radial rows of procambial cells are produced first in the middle of the bundle and then extend toward either flank. ×240.

of the confronting leaf, leaf 14 in this case (fig. 10). Adjacent to these vacuolated cells the two leaf strands characteristic for *Ginkgo* are just recognizable. Since these two leaf strands have different origins and different vascular connections below, as will be shown in the second paper, they do not ordinarily possess the same degree of development at any level. Thus, the narrow procambial cells (*pc*) resulting from scattered, periclinal cell divisions are more evident in the right than in the left strand.

In leaf 2, one plastochrone older, two leaf strands are now more clearly evident. This older and larger leaf shows ab- and adaxial vacuolated regions (fig. 14, *abv, adv*), confluent around the flanks of the leaf strands as well as between them. The strands themselves provide evidence of their development. The scattered periclinal divisions continue; they are here supplemented by peripheral anticlinal divisions, especially on the flanks, thus extending the circumferential dimension of the strands at the expense of the adjacent, recently vacuolated cells.

In leaves 3 and 4 (fig. 15, 16), further changes are apparent. Polarized periclinal divisions take place first in the median portion of each trace (fig. 15), producing a radial row of cells. Successive

rows then develop towards either flank of each trace, thereby increasing the number of radial rows, as seen in leaf 4 (fig. 16). As will be shown in the second paper, a band of cells across these rows becomes the locus for further procambial divisions and eventually for the beginning of the fascicular cambium. At the stages of development shown in leaves 3 and 4 (fig. 15, 16), the strands are delimited in transverse and longitudinal section by a sheath of parenchymatous cells, compressed somewhat circumferentially by the increase in lateral and in radial dimensions of the procambial strands.

The first procambial cells in a young leaf primordium are formed by longitudinal divisions in a median group of the meristematic cells comprising the primordium. At this stage of development, except for the narrower diameter of the procambial cells, there is great similarity between these and the other cells of the primordium, whether seen in transverse or longitudinal section. A longitudinal section through such a young leaf primordium, however, shows that these procambial cells are of gradually increasing length when followed downward into the older parts of the strand in the axis below. By such elongation these cells keep pace with the growth of the pith and cortex. The procambial strand can be followed obliquely downward to its older regions where it becomes confluent with the flanks of the procambium of a lower leaf trace. Serial longitudinal sections show the continuity of the procambium around both sides of the leaf gap.

It is possible, from the established phyllotaxis, to predict accurately the position of the next leaf primordium to be produced (leaf-1) before it appears as a detectable protuberance on the stem apex. Figure 9 is a diagram of a transverse section cut just above the shoot apex, from a terminal short shoot collected in late July, during the setting of buds. The phyllotaxis was determined as previously indicated. This tip was then cut into serial longitudinal sections. From a study of these sections, the positions of the youngest leaves were then determined and accurately plotted into the established "spirals" of the 5/13 (or 5 + 8) phyllotaxis (fig. 9). The position of leaf -1, or the next leaf to appear on the apex, must appear in the same "spiral" as leaf 5 and the confronting leaf 13 (fig. 10). With the locus of leaf -1 reasonably well established, the next step is to determine whether the procambium can already be identified in this area of leaf initiation.

In *Ginkgo,* with its two traces to each leaf, one procambial strand is confluent with the strand of the next lower leaf in the same phyllotactic "spiral"; one, with a branch from an adjacent "spiral." In this case, leaf -1 will receive one strand from leaf 5 and one from leaf 8 (fig. 11). The latter (*-1a,* fig. 11; *pc: -1,* fig. 12) has developed acropetally, occupying a path which cuts obliquely and laterally across the buttress of leaf 5.

As one follows acropetally a developing procambial branch such as -1a, the elongate, narrow cells become shorter and shorter towards the apex until in the peripheral subsurface layers one recognizes the procambial region only by the frequent longitudinal divisions and the narrow diameters of the recently divided cells (fig. 12, *pc: -1*). In fact, were it not that these divisions were at the upper end of the already recognizable procambial strand, they could not easily have been identified for what they seem to be, for no foliar primordium exists at this time as a counterpart for their development.

After studying many cauline apices of both long and short shoots, collected in midsummer, it became apparent that the acropetal extension of the presumptive procambial strand can be pictured by the pattern of active longitudinal divisions or those just completed (fig. 12, taken from the region of the broken line in fig. 10). That it is possible to follow the extension of this presumptive procambial strand because of the pattern of active longitudinal divisions, and that thereby the locus for the development of leaf -1 can be predicted, a locus which coincides with that determined by plotting the phyllotaxis, seems particularly significant. Moreover it has been possible in repeated instances to follow the pattern of development for each strand of the double leaf trace, one on either side of the leaf gap. Thus a series of longitudinal sections also gives indication of the origin of the second trace (*-1b*) of leaf -1 from leaf 5 (fig. 11).

The pattern of procambial development described above for the terminal short shoot occurs in all shoot apices studied, whether short shoots, long shoots, or those of transitional nature. One must emphasize that the stages of procambial formation which precede the formation of leaf primordia were found only at the time of developing buds.

Discussion.—*Ginkgo,* whether in long or short shoots, does not show seasonal variation in the shoot apex comparable to that described for *Ephedra altissima* (Gifford, 1943) or *Torreya californica* (Kemp, 1943). Although there are seasonal variations in shoot development or shoot expression in *Ginkgo,* such changes are reflected in the derivatives from the apex and not in the structure or configuration of the apex itself.

The first obvious change in the peripheral subsurface layers is the progressive acropetal vacuolation of those portions subjacent to successive developing leaf primordia. Wetmore (1943), in a review of leaf-stem relationships, has pointed out in a number of angiosperms and gymnosperms that vascular and cortical patterns are generally correlated with the formation of leaves at the apex; the pith by contrast seems to belong to the axis.

The zone of central rib meristem in the shoot apex quickly produces, independently of the cortex, a broad, rapidly maturing pith, as evidenced by the early appearance of druses, especially in short shoots, within three or four cells of the rib meristem. Fankhauser (1882) described the intercellular spaces and the resin ducts of the pith in

Ginkgo as terminating only a millimeter below the surface of the apex; a quarter of that distance would be more nearly correct. The pattern of pith development is determined in considerable measure by the extent of rib meristem activity. This activity is reflected in its derivatives unless the initial pattern is lost by subsequent changes resulting from cell enlargement or cell separation.

Representatives of seven genera of the cycads which have been studied recently (Foster, 1939, 1940, 1941, 1943; Johnson, 1939, 1944) show not only a marked degree of resemblance to *Ginkgo* in the zonal pattern of the shoot apex but also a comparable rib meristem development of the pith. Variations in the rib development between different apices of the same species suggest again that the difference between pith development in long shoots and in short shoots of *Ginkgo* is quantitative rather than qualitative.

Koch (1891), Barthelmess (1935) and Kaplan (1936, 1937) made the generalization that, in the axis of gymnosperms, pith matures early but the entire cellular cylinder surrounding the pith remains more or less homogeneous to constitute a peripheral meristematic zone. This zone later separates into an outer vacuolated cortex and an inner residuum of meristem in which the procambial strands develop. Barthelmess described for gymnosperms an early beginning of the pith above the origin of the first leaf primordium, while the vacuolated cortex is not recognized higher than the buttresses of the fourth to the seventh primordia. Yet he states, in seeming contradiction, that, in *Ginkgo*, pith and cortex develop uniformly and simultaneously. Kaplan (1936) pointed out that the stem apex in *Ginkgo* is not cone–shaped but keel-like; consequently the places of attachment of older leaves lie above those of younger leaves. Further, he stated that although the youngest leaves are surrounded by older and more developed buttresses, the wide and cone-shaped pith, as he recognized it, begins just below the apical meristem but higher than the first leaf. He also pointed out that the leaves at first form much ventral (adaxial) parenchyma and only later form dorsal (abaxial) parenchyma.

In evaluating these observations on *Ginkgo* by Barthelmess and Kaplan, careful consideration must be given to the stage of development under discussion. The apex of *Ginkgo* is broad and flat—certainly only broadly keel-shaped—and the internodes in this portion of the axis are so short that it is impossible, without detailed study of serial sections, to follow progressive changes in the apical meristem above the pith or cortex. Moreover the cells of all meristems possess vacuoles, as shown by Bailey (1930) for the cambium, and by Zirkle (1932) for primary meristems; hence, any difference between the vacuolation in meristems and that in their derivatives is one of degree. It is also pertinent to point out here that the term "parenchymatization" as used by Kaplan and others seems to refer to the process of so-called maturation as expressed by increased vacuolation. It is quite true that successive transverse sections of *Ginkgo* from the stem apex downward show vacuolation of cells in the mature pith region to be evident early, and to proceed independently of the vacuolation of cells in the cortical region at the same level. Yet in no case did the pith appear higher than the first leaf. Both Barthelmess (1935) and Kaplan (1936) must have included in the pith of *Ginkgo* the mother-cell zone of the apex.

It seems clear that in *Ginkgo* progressive vacuolation can be followed higher in the cortex than either Barthelmess or Kaplan has indicated for the pith. That is, the first marked development in the apex becomes evident in the derivatives of the outer peripheral subsurface layers with the appearance of highly vacuolated cells in the area where the next leaf is to be initiated. These cells are in continuity with similar vacuolated cells in the gap region of the confronting leaf and eventually will also be continuous with the abaxial vacuolation of the new leaf as it develops. Slightly later, from the developing pith, further vacuolation proceeds continuously and acropetally across the leaf buttress and into the adaxial side of the leaf primordium. Eventually in each leaf primordium the ab- and adaxially vacuolated regions become confluent around the procambial strands. That is, as the plant grows, the apical meristems, by progressive development, add increments to the stem apex in which the growth patterns are developed in continuity with those established below.

The procambium develops acropetally along the inner portion of the peripheral subsurface layers, that is, between the ab- and adaxially vacuolated regions just described. Barthelmess (1935, p. 212) describes three characteristics for the early procambial cells of conifers: (1) the elements are relatively long though possessing the smallest cross-sectional diameter of any cells in the transverse section at that level; (2) the cytoplasm of those cells stains more darkly than that of the relatively highly vacuolated neighboring cells of the pith and cortex, and almost the whole cell is filled by the dark-staining nucleus; and (3) these cells in transverse view are arranged in short radial rows as characteristic for the elements of mature bundles in conifers.

In considering these points of Barthelmess, it is evident that in the apical region the first cells of the procambium are hardly distinguishable, other than by position, from the meristematic cells characteristic of the apex. It is true that longitudinal divisions in the apical cells do produce the small, transectional diameter of the procambial cells, the first noticeable sign of their formation. Since the procambial strands of *Ginkgo* develop obliquely toward the apex, a transverse section cuts the strands on the bias and the small diameter is usually somewhat obscured. In longitudinal section, the short, scarcely identifiable procambial cells at

the upper end of the developing procambial strand are in vertical continuity with progressively longer cells in the strand below. By such elongation, these cells keep pace with the transversely dividing cells of pith and cortex. The cytoplasm of the youngest procambial cells is not markedly different from the other cells in the peripheral subsurface layers at that level. However, the small transverse diameter and also the later progressive vacuolation of the flanking cortical and pith cells is responsible for the apparent greater density of protoplast in these procambial cells. After elongation, the protoplasts of procambial cells may become as highly vacuolated as those of the cells of pith and cortex. Finally, the short radial rows of procambium, in *Ginkgo* at least, are formed only after the procambium is well established and easily discernible within the meristematic bands delimited by ab- and adaxial leaf vacuolation. Only then do polarized periclinal divisions appear in the center of the bundle and extend progressively toward either flank.

In the light of the present investigation, these characteristics of coniferous procambium, as suggested by Barthelmess, clearly are not applicable as criteria for recognizing the early procambium of *Ginkgo*.

The pattern of procambial development in *Ginkgo*, once established, can be followed continuously and acropetally into the youngest leaf primordium. Koch (1891) pointed out the acropetal differentiation of procambium in gymnosperms. Barthelmess (1935) and Kaplan (1937) described basipetal differentiation of leaf traces. More recent work on certain conifers (Cross, 1942; Crafts, 1943a, 1943b) has pointed out the continuous acropetal development of procambium in those species.

Since the results summarized here have been duplicated in many tips of both short and long shoots in *Ginkgo*, it seems fair to state that procambial development to any leaf primordium must originate from some procambial strand or strands already present in the lower axis and develop acropetally to each new primordium. From this study, as Esau (1942) found might well be the case in *Linum perenne* L., it is suggested that the procambium is established in the apex below the area of leaf initiation before the leaf primordium is a recognizable protuberance. The recent report by Sterling (1945) of procambial strands being established in the stem apex of *Sequoia sempervirens*, not only for the next presumptive leaf, but for the first six such leaves, is worthy of comment at this time.

SUMMARY

Two well-defined types of shoots, *long shoots* and *short shoots*, are recognized in *Ginkgo biloba* on the basis of gross morphology. The former type has its leaves separated by internodal intervals along the stem, the latter has no appreciable internodes so that the leaves appear crowded at the apex of the stem. Though all buds, terminal and lateral, produce short shoots each spring, a varying number of these will change to long shoots in the course of the season. Conversely certain long shoots will become short shoots.

Shoot expression is correlated with quantitative differences in the steps of development from the apical meristem rather than differences in the configuration of the meristem itself.

Localized activity in the peripheral subsurface layers of the shoot apex gives origin to leaf primordia. The cortex is formed as the abaxially vacuolated cells of successive leaf primordia and their buttresses surround the stem apex. Both ab- and adaxial vacuolation of leaf primordia proceed acropetally. Because of differences in cell division and enlargement, the cortex of a short shoot is radially broad and spongy while that of a long shoot is narrow and more compact.

The pith is formed, independently of the cortex, by the activity of a pith rib meristem. The amount of pith produced is largely determined by the degree of activity in this rib meristem, corresponding to the amount of shoot growth. Much meristematic activity exists in the long shoot, little in the short shoot. The pattern of pith development affords strong evidence to suggest that the short shoot is the characteristic type for *Ginkgo* at the present time.

The two procambial strands of a leaf develop continuously and acropetally from definite procambial strands already present in the lower axis. The evidence suggests that the procambium is established below the area of leaf initiation before the leaf primordium is developed.

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CULTIVATION OF EXCISED STEM TIPS OF DODDER IN VITRO [1]

Shih-Wei Loo

EVIDENCE HAS been accumulated which shows that the growth of the excised stem tips of asparagus (*Asparagus officinalis* Linn.) can be maintained indefinitely *in vitro* in a synthetic medium in the light (Loo, 1945a, 1945b, 1946). Since asparagus is an autotrophic plant, it would be of interest to study the nature of the growth of excised stems of an heterotrophic plant. A series of experiments, therefore, was carried out with dodder (*Cuscuta campestris*), a parasitic plant. The growth of dodder, unlike that of asparagus, is entirely dependent upon a supply of food materials and presumably of growth factors from its host plant.

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The writer wishes to express his sincere appreciation to Professor James Bonner for his inspiration and critical advice throughout the whole course of the work. He is especially indebted to Dr. C. W. Bennett of the Division of the Sugar Plant Investigations of the Bureau of Plant Industry at Riverside for the supply and identification of dodder seeds and also for his kindness in reading the manuscript. This investigation was carried out at William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California.

The dodders have attracted the interest of botanists because of their peculiar morphological and physiological adaptations to a parasitic mode of life. A general study of their structure and behavior has been reported by Palm (1827), von Mohl (1827) and Koch (1874, 1880). The formation and histological structure of the haustoria of dodder in relation to the host have been extensively investigated by Peirce (1893), Thoday (1911), Zender (1924), Schumacher (1934) and Schumacher and Halbsguth (1938). The physiology of *Cuscuta* has been studied by Uloth (1860), Peirce (1894), Mirande (1901) and Spisar (1910). Very recently, this plant has been used for the study of transmission of virus by Bennett (1940, 1944), Johnson (1941a, 1941b) and Kunkel (1943a, 1943b).

A general review of the literature on the culture of stem tips has been given in previous papers (Loo, 1945a, 1945b) and will not be reiterated here.

MATERIALS AND METHODS.—Dodders are non-chlorophyll-bearing, parasitic seed plants with twining stems. The leaves are reduced to minute scales. Flowers are small, about two to three mm.

long, calyx and corolla 5-lobed. The thread-like stems twine around the stems or leaves of the host. Haustoria develop on the stems of the parasite where they make contact with the host. The haustoria penetrate into the vascular tissue of the host and absorb nutrients there. For the particular species, *C. campestris,* used in this investigation, the

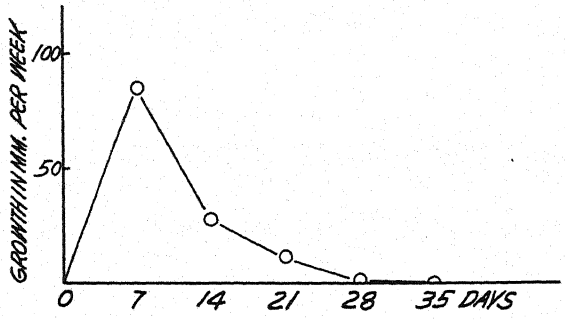


Fig. 1. Growth of normal dodder seedlings in culture (Exp. B-17).

following appear to be the best of the hosts tested by Dr. C. W. Bennett (personal communication):

Tobacco
Tree tobacco (*Nicotiana glauca*)
Sugar beet
Pokeweed (*Phytolacca americana*)
Jimson weed (*Datura stramonium*)
Tolgaucha (*Datura meteloides*)
Various species and varieties of clover and alfalfa

The technique, procedure and nutrient used for the culturing of dodder stem tips were essentially the same as those used for asparagus (Loo, 1945a) except for a few minor modifications which will be described later. As soon as the seedlings reached a length of 2 to 5 cm., the stem tips were cut off and transferred to culture tubes containing various nutrients and then grown under different cultural conditions. Culture experiments with dodder are difficult because of the very low percentage of germination of the seeds. Usually, less than 5 per cent of the seeds germinate and sometimes less than 2 per cent. Hence, a very large number of seeds must be used for even a small experiment. Every effort was made to improve seed germination but without success.

Growth of normal seedlings.—In the first experiments whole seedlings about 2 to 3 cm. long were transferred to culture tubes containing either liquid medium or one per cent agar medium made with Hoagland's solution (Hoagland and Arnon, 1938), plus two per cent sucrose. These cultures were placed in diffuse light. The growth rate of the intact seedlings was very high in the first week, but dropped abruptly in the second week, and decreased to almost zero at the end of the third week. An average total growth of 100 to 150 mm. was obtained during a period of five weeks. Figure 1 is a graph showing the average growth of normal seedlings in liquid and agar media. It should be pointed out that the root of dodder usually does not develop well in the field. This was also true in culture; the rudimentary root appeared more or less like a small club and appeared to be without a cap. The tissues of the seedlings underwent differentiation, but microscopic examination disclosed no lignified cells in the central cylinder. This is in agreement with the results of Peirce (1894) who studied the young seedlings grown in pots.

Effect of light conditions on the growth of excised stem tips.—In further experiments, stem tips were excised from the seedlings transferred to fresh medium. This transfer was repeated at intervals. The growth of excised dodder stem tips was investigated under different light conditions. The results of two such tests are given in tables 1 and 2.

Table 1. *Growth of excised stem tips of dodder in 0.5 per cent agar basal medium + minor elements, 0.1 gm. per liter of yeast extract, 4 per cent sucrose at 26°C. (Exp. B-22-1).*

Transfer number	Transfer interval, days	Average growth rate in mm. per day	
		Diffuse light	Dark
1	8	9.0	...
2	16	2.4	4.9
3	25	0.5	0.8
4	38	0.4	0.7
5	29	alive[a]	dead

[a] One tip produced 2 buds, another several buds.

Table 2. *Effect of different light conditions on the growth of excised dodder stem tips in 0.5 per cent agar basal medium + minor elements, 0.1 gm. per liter of yeast extract and 4 per cent sucrose (Exp. B-29).*

Treatment	Average growth in mm. (10-day transfer interval)		
	1st transfer	2nd transfer	3rd transfer
Dark	77.8	29.9	22.5[a]
	76.5	31.0	...
Diffuse day light.....	78.5	32.9	27.0
	66.2	33.9	38.9
	88.0	34.0	22.1
8 hours artificial light..	71.0	39.6	25.9
16 hours artificial light..	66.5	37.0	15.9
24 hours artificial light..	95.3	29.6	11.8
	93.5	31.2	14.5

[a] This is an exceptional case in which dodder stem tips made considerable growth in dark in the third transfer.

Regardless of the light conditions, the growth rate of the excised stem tips followed the same pattern as that for normal seedlings described in the previous section. In general, the various light conditions tested did not show significantly different

effects in the first two or three weeks. However, subsequent growth can be secured only in the presence of light. In the dark, the growth of stem tips usually stopped entirely after two or three transfers (about two to three weeks) and the tips finally died. In the light the stems remained alive even through the fifth transfer. This shows clearly that light benefits the growth of excised stem tips of dodder.

In light, after four or five transfers, the growth of the stem tips remained at a scarcely measurable level for a long time. Since the agar medium tended to dry up, the nutrient was renewed by transferring the whole tip to fresh medium from time to time. During this period, the tips increased in thickness and apparently initiation of lateral buds occurred. Eventually, the growth of the culture was resumed from these lateral buds. Figure 3 is a photograph showing the emergence of several lateral branches developed from such a culture. Subcultures of the lateral branches were made which will be described later.

However, there were some differences among the tips grown under various light treatments. As shown in table 2, the growth of stem tips in the first seven to ten days was not greatly affected by light, except under continuous illumination. It was observed that the excised stem tips grown in the light usually became green in color at the very tip. As illustrated in figure 2, a considerable number of chloroplasts

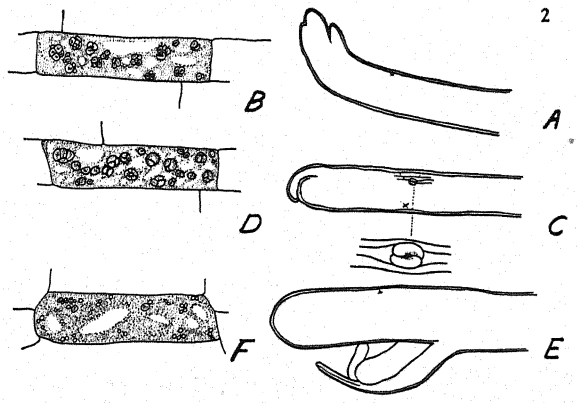


Fig. 2. Stem tips of dodder (Exp. B-29): A, excised stem tip grown in the dark; B, a cell from (A) showing leucoplast with starch grains; C, excised stem tip grown in the light, with a stomate; D, a cell from (C) showing chloroplasts with starch grains; E, stem tip of normal seedlings grown in greenhouse; F, a cell from (E) showing granules which stained brown with iodine. (B, D and F drawn under 6 x 90 × and reduced to ⅓).

was developed. An iodine starch test indicated that starch synthesis had apparently taken place in these stem tips. This is in contrast to field plants and excised stem tips grown in the dark which were either yellow or white in color. Such plants contain only leucoplasts and a small number of starch grains.

Effect of sugar on the growth of excised tips.—The effect of additions of sucrose to the basal medium over a concentration range of zero to twenty per cent was tested upon the growth of excised dodder stem tips. The results of one such experiment are summarized in table 3. The excised

Table 3. *Effect of sugar on growth of excised dodder stem tips grown in 0.5 per cent agar basal medium + minor elements, 0.1 gm. per liter of yeast extract, in continuous light at 26°C. (Average of 12 tips, Exp. B-25-1.)*

Culture number	Per cent sucrose	Average growth in mm. 1st transfer, 5 days	2nd transfer, 9 days	3rd transfer, 10 days
1	0	83.5	37.4	0.0
2	1	85.0	38.0	12.2
3	2	72.5	34.4	12.7
4	4	86.0	38.0	15.4
5	6	86.6	35.0	11.1
6	8	74.0	41.0	12.2
7	10	82.5	41.2	13.5
8	15	72.5	36.2	16.2
9	20	74.0	39.6	14.1

stem tips grown in the light made a considerable growth in the first two weeks even without an external supply of sugar, but thereafter they ceased growth and finally died. The growth in various sugar concentrations was, on the whole, about the same, although 4 per cent sugar seemed slightly beneficial. It is interesting to note that the growth of excised stems in 20 per cent sugar was quite normal and similar to that in lower sugar concentrations.

Formation of lateral branches and flowers on excised stem tips.—As previously mentioned, stem tips stop growing after a period of four to five weeks and remain in this condition for ten to eleven weeks. During this latter period, it was necessary to renew the nutrient medium by transferring the whole tips to fresh media from time to time. While in this condition of barely discernible growth, the tips nevertheless increased in thickness and buds were formed. In one such experiment (table 4) a culture was started on November 24, 1944. After the fourth transfer on January 12, 1945, the growth had nearly ceased and the culture medium was changed several times by transferring the whole tips to fresh medium. On March 20, 1945, growth was resumed by the elongation of lateral buds. The lateral buds were cut off and subcultured. The growth rate was quite comparable to that of the initial tips as shown in table 4. Figure 3 is a photograph showing one such stem tip which resumed growth and produced several lateral buds.

Another very interesting phenomenon is the formation of floral organs in excised dodder stem tips as indicated in table 4 and figure 4. This culture was also started on November 24, 1944. The growth of the tip followed the already described pattern. On

February 19, 1945, two small buds were identified as flower buds. Figure 4 is a photograph of these two flowers taken on April 21, 1945. The picture shows that one flower was fully opened and the other remained a flower bud. Throughout the whole period of cultivation, the excised stem tip was grown in diffuse light at 26°C. in a 0.5 per cent agar medium

TABLE 4. *Growth of original excised stem tips and excised lateral buds in 0.5 per cent agar basal medium + minor elements, 4 per cent sucrose, 0.1 gm. per liter of yeast extract in diffuse light at 26°C. (Exp. B-22-2).*

Transfer number	Transfer interval, days	Date	Average growth rate in mm. per day
		Original excised stem tips	
1	8	Nov. 24, 1944	9.0
2	16	Dec. 2, 1944	2.4
3	25	Dec. 18, 1944	0.5
4	38	Jan. 12, 1945	0.4
5	29	Feb. 19, 1945	alive[a]
		Excised lateral bud	
1	8	Mar. 28, 1945	8.5
2	9	Apr. 6, 1945	3.9
3	10	Apr. 16, 1945	2.1
4	13	Apr. 29, 1945	0.9

[a] One tip produced 2 buds which were later identified as flower buds, another produced several buds.

containing mineral salts, minor elements, 4 per cent sucrose and 0.1 gm. per liter of yeast extract. The nutrient medium was changed every two to three weeks. This is probably the first instance where flower buds have been observed on excised stem tips. The formation of floral organs has never occurred in the case of excised stem tips of asparagus which have been kept growing *in vitro* for a period of twenty-two months (Loo, 1945a, 1945b, 1946).

DISCUSSION.—From the results described above, it is apparent that light is favorable for the continuous growth of excised dodder stem tips. A considerable amount of chlorophyll and many plastids were formed in the light. This is apparently much more chlorophyll than is contained in the normal plant. MacKinney (1935b) identified traces of chlorophyll in dodder by light adsorption measurements after large scale extraction. This minute amount of chlorophyll in the normal plant is greatly overshadowed by the carotenoids and is not detectable by ordinary microscopic examination. The existence of chloroplasts in young dodder seedlings has been observed also by Temme (1883) who was able to demonstrate the evolution of oxygen, an indication of photosynthesis. This was regarded as an abnormal phenomenon by Peirce (1894) who showed that the development of chlorophyll in this parasite is always a consequence of insufficient food. He reported that dodder plants grown on thriving flax plants in the field were vigorous and developed a typical, pale orange coloration, while those grown on flax in the laboratory were small, weak and had a decidedly green color. It has since been shown by MacKinney (1935a, 1935b) that the normal yellow-orange coloration is due to an unusually high content of carotenoids. In some species of dodder (*C. subinclusa* and *C. salina*) he has found as much as 20 to 25 mg. of γ-carotene per kilogram fresh weight in addition to α- and β-carotene (12.5 mg.), rubixanthine (2 to 3 mg.), lycopene (4 to 6 mg.) and a trace of chlorophyll. Peirce cut off the tips of dodder (8 cm. long) and attempted to grow them in water or in a decoction of host plants. For this purpose he cultured *C. glomerata* and *C. europaea* on extracts made from *Impatiens* and *Chrysanthemum*. After 48 hours, all tips became nearly as intensely green as the leaves of their host plants. However, within three days they ceased to grow and finally all died. He concluded that "when occasion demands, the *Cuscuta* can develop a very considerable quantity of chlorophyll, . . . (It) is a most interesting confirmation of the hypothesis that this genus of plants has become parasitic within comparatively recent times." Hence, the development of chlorophyll in dodders is an indication of their normal ancestral life before the adoption of a parasitic mode of life.

It is apparent that, with a sufficient supply of food from the host, dodder plants do not need to synthesize their own food and consequently no chloroplasts are produced. However, it seems probable that chloroplasts do play an important role in maintaining the excised stem tips in culture for more than five months, with the subsequent formation of lateral buds and flowers.

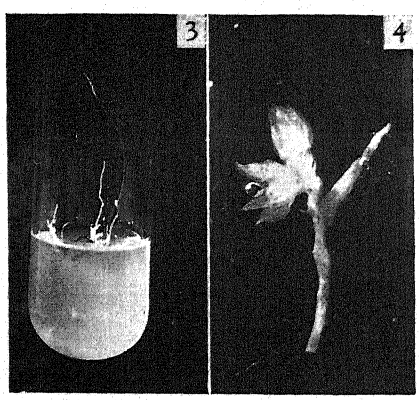


Fig. 3–4.—Fig. 3. Lateral buds developed from excised dodder stem tips *in vitro* (November 24, 1944 to April 27, 1945, photograph ×6/11.—Fig. 4. Flower and flower buds developed from excised dodder stem tips *in vitro* (November 24, 1944 to April 21, 1945, photograph, ca. 5×).

Of numerous supplementary substances tested, only sugar was shown to be required for the growth of dodder stem tips. Among the others, yeast extract had a favorable effect in the first transfer, but this effect declined through subsequent transfers. It seems that yeast extract is not necessary for the

growth of excised dodder stem tips. Since dodder is a parasitic plant which absorbs its nourishment from its host plant, it was of interest to see if the growth of excised stem tips could be maintained by feeding them with an extract from a host plant. However, lyophylized whole cytoplasm[2] prepared from the leaves of various host plants neither stimulated nor retarded growth. There are several possible reasons why this latter substance was without effect. Perhaps the excised stem tips were unable to absorb the substances contained in the whole cytoplasm because of the absence of haustoria. These absorbing organs have never been observed in excised stem tips *in vitro*. Attempts were made to induce the formation of haustoria on excised stem tips and were without success. According to Zender (1924), Schumacher (1934) and Schumacher and Halbsguth (1938) haustoria are directly linked with the phloem tissues of the host. Histological studies showed clearly the connections between the vascular tissues of the two plants, so that the parasite is able to absorb its nutrient materials directly from the vascular system of the host plant. Schumacher (1934) traced the migration of food materials from the host to the parasites by the use of fluorescein.

In nature, after germination, dodder seedlings continue to grow only after the haustoria have secured connections with the vascular tissues of the hosts. It is probable then, that haustoria are needed for the absorption of the substances contained in cytoplasm. There is no doubt that the stem tips do absorb nutrients from the agar medium since they were able to increase in thickness and produce lateral buds and flowers and are apparently able to absorb sucrose. It is also possible that the stem tips require some substances produced only in the living tissues or in the roots of the host plant, such as caulocaline (Went, 1938).

While the simple nutrient medium containing sugar, yeast extract and minerals used is probably not optimal for the growth of dodder stem tips, it should be emphasized that it has been possible to maintain excised stem tips of dodder *in vitro* for more than five months without the formation of leaves or roots. The culture is still growing at the time of reporting these data. Furthermore, the excised stem tips were able to form lateral buds and floral organs in the same medium without the addition of other growth-promoting substances or any external source of supply of "flower hormone."

[2] The writer wishes to thank Dr. Sam Wildman for his help in the preparation of the lyophylized cytoplasm.

SUMMARY

It has been possible to maintain excised stem tips of dodder *in vitro* for a period of five months through several transfers.

The excised dodder stem tips produced lateral buds and flowers in a simple medium without the formation of leaves and roots. This is probably the first case where floral organs have developed on excised stem tips *in vitro*.

A considerable amount of chlorophyll is produced in the excised stem tips of dodder and probably plays an important role in maintaining the growth of the culture. No indication of the formation of haustoria on the excised stems was observed.

Sugar was found to be essential for the growth of dodder stem tips. Other supplementary substances tested including lyophilized whole cytoplasm from the leaves of host plants failed to promote growth.

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DEVELOPMENT IN STERILE CULTURE OF STEM TIPS AND SUBJACENT REGIONS OF TROPAEOLUM MAJUS L. AND OF LUPINUS ALBUS L. [1]

Ernest Ball

THEORETICALLY, the shoot apex of higher plants should prove the ideal object for experimental morphology. It produces by its activities tissues from which all the vegetative parts of the shoot are differentiated. The problems of differentiation, or maturation of tissues down the plant shoot, have been subjected to few physiological studies. Higher plants, with the young, embryonic tissues at the stem tip, and progressively more differentiated or mature tissues in the subjacent regions, are excellent material for *in vitro* studies.

Little consideration in the published work has been given to such significant morphological characteristics as polarity, potentiality for development of tissues during maturation, and correlation. The great theoretical interest of such data prompted the writer to carry out several series of sterile cultures of shoot apices (with little additional tissues), and of subjacent tissues undergoing maturation.

Rechinger (1893) grew isolated buds of *Populus nigra, Fraxinus ornus* and other plants in sand without nutrient materials. Some basal callus and a slight expansion of upper parts occurred, but no roots were formed. Goebel (1908) grew isolated buds of *Ceratopteris thalictroides* in moist peat and found that they produced leaves much smaller and simpler morphologically than those of normal plants. Robbins (1922) apparently first grew shoot tips in sterile conditions with sugar. He grew stem tips (1.75 to 3.75 cm. long) of pea, corn and cotton in the dark in agar with glucose. The tips produced shoots with small chlorotic leaves and numerous roots. White (1933) grew stem tips (0.1 mm. or less in length and width) in hanging drops of culture medium. They lived up to six weeks. Some showed differentiation, i.e., they produced foliar or flower primordia, and others merely enlarged. White considered that the enlargement usually resulted from cell divisions and not from hypertrophy. No complete plants (stem, leaf and root) were achieved. La Rue (1936) grew segments that included cotyledon bases and plumular meristems or shoot tips of *Taraxacum officinale, Chrysanthemum leucanthemum, Lactuca canadensis, Lycopersicum esculentum,* and buds from leaf bases of *Radicula aquatica* (mature plants) in sterile agar and liquid media. Inocula were less than 1 mm. long and usually less than 0.5 mm. wide. Roots were regenerated basally on these buds and the shoots produced normal plants. Segelitz (1938) grew shoot tips of corn (1–4 cm. long) on a sterile agar substrate. The tips over 2 cm. long rooted in the dark, but those under that length rooted only in light. Large plants were grown from these cultures. Smith (1940) grew stem tips (0.55 to 4.0 mm. long) of *Helianthus annuus* on agar in White's solution. Studies were made of the effects of various accessory growth factors in the medium on the rates of growth. Loo (1945) grew stem tips (5 to 10 mm. long) of *Asparagus officinalis* in sterile culture through twenty successive transfers. He considered these cultures to be the first true stem (organ) cultures that have been achieved and to be comparable with the organ cultures of excised roots described by White, Robbins, Bonner and others. De Ropp (1945) reported that the growth of excised stem tips of rye embryos was confined to the first leaf. When roots were produced, which was rarely, growth was more normal.

Much controversy has occurred in the literature concerning the capacity for growth and development of vegetative plant cells. It was the purpose of the writer to obtain evidence on this matter by comparing the growth *in vitro* of the apical, meristematic tissues with that of the subjacent maturing tissues of the shoot.

Also, since the respiratory rates of the tissue pieces used in these experiments had been previously determined (Ball and Boell, 1944), it was considered worth while to find whether a relationship exists between rate of respiration and developmental capacity.

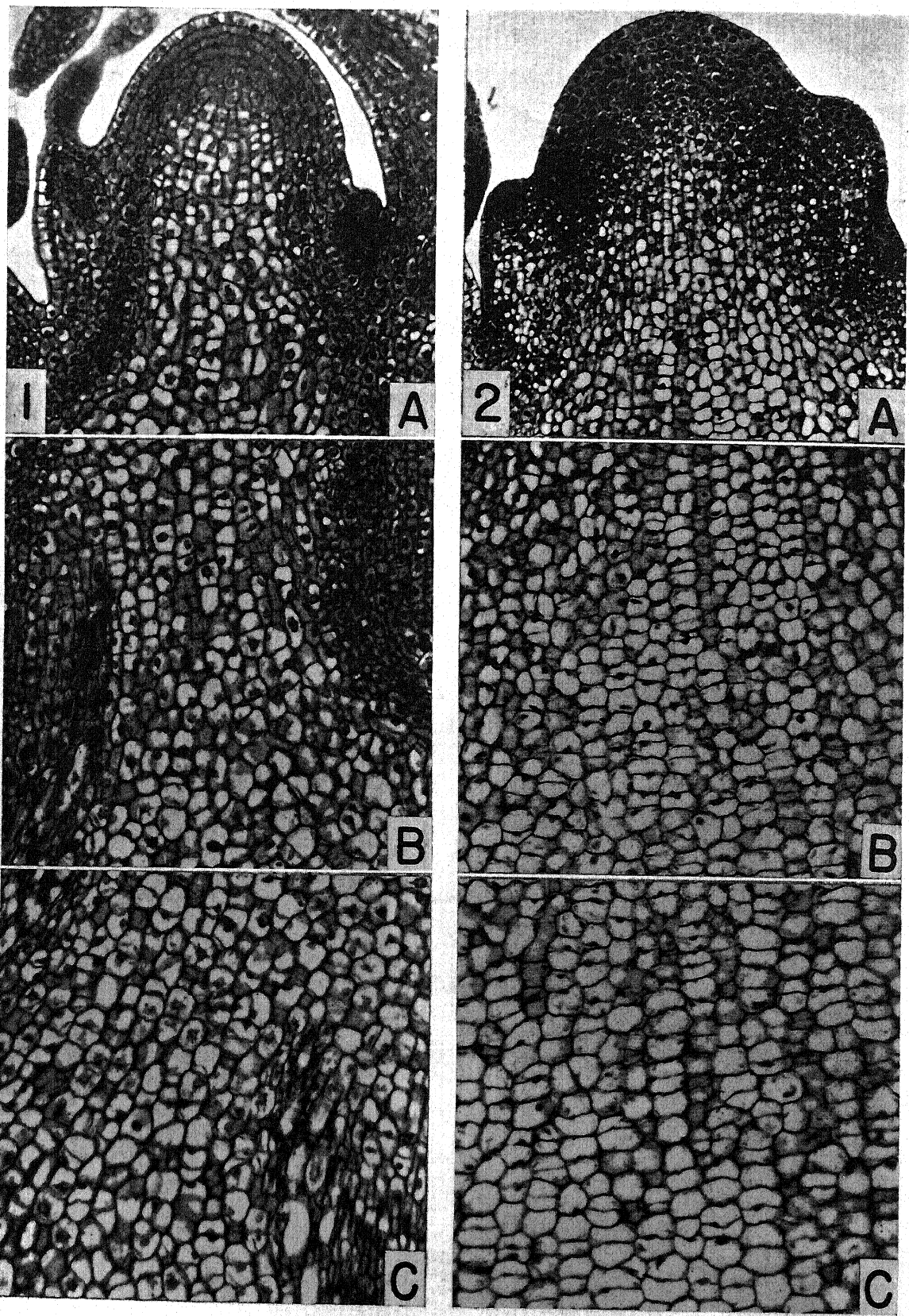
Further, these cultures lent themselves well to studies of the polarity of the buds and subjacent maturing tissues. The polarity of buds is especially poorly understood.

MATERIALS AND METHODS.—Seedlings thirty days old were used. These plants were purely vegetative and showed no indication of flowering. They appeared to be past the stage when the major supplies of foods, growth factors, etc., were derived from the seeds. Each had produced eight to ten mature foliage leaves. Undoubtedly most of their organic nutrition was provided by the synthetic activities of these leaves.

The shoot apex of *Tropaeolum majus* L., var. Dwarf Golden Globe, is small; average dimensions are 55 μ high, 140 μ thick (fig. 1). This dome of meristemattic tissue, the surrounding primordia and the subjacent tissue are devoid of chloroplastids and display a whitish-yellow color. The phyllotaxis is helical. Axillary shoot apices are formed in connection with each foliar primordium. The growth of the axillary meristems is inhibited only during early seedling stages and the plant soon shows a much branched character. This plant thus displays no pronounced apical dominance. Tissues subjacent to the shoot

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1
A
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A
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C
C

apex mature into pith that usually remains alive and intact as long as the stem survives. Only occasionally do central cells of old pith break down. *Tropaeolum majus* has pronounced regenerative powers and easily forms adventitious roots and buds.

The shoot apex of *Lupinus albus* is larger; average dimensions are 81 μ high and 250 μ thick (fig. 2). Chloroplastids are abundant in the cells of the shoot apex as well as in the surrounding foliar primordia and subjacent tissues; all of these regions are bright green. The phyllotaxis is helical. Axillary shoot apices are formed in connection with most foliar primordia, but they rarely grow to form lateral shoots. The terminal shoot apex exhibits complete dominance over the lateral shoot apices. Axillary buds never sprout during the vegetative growth of the plant except when the terminal bud is removed. Tissues subjacent to the shoot apex mature into pith that soon dies and undergoes disintegration, leaving the mature stem hollow. *Lupinus albus* has little regenerative power and seldom forms adventitious roots and buds.

The two plants used in these experiments thus differ anatomically and physiologically. The experiments gave promise of information on the effects of excision and sterile culture on polarity, developmental capacity, correlation, and nutrition for plants of two different anatomical and physiological "types."

Both *Tropaeolum* and *Lupinus* are advantageous objects for the dissection and excision of small stem tips and small bits of subjacent tissues. The bud of *Tropaeolum* is loose, and the shoot apex may be reached with little disturbance of older leaves. The bud of *Lupinus* is more compact, since there are sheathing leaf-bases, but the petioles of the older leaves may be bent outward to expose to view the dome of meristematic tissues. After the shoot apices were exposed, a very small knife (made by welding a sliver of very thin safety razor blade to a small sewing needle) clamped in a dissecting-needle holder was used to excise the tissues. Under a calibrated, binocular dissecting microscope, the terminal shoot apex with its three youngest foliar primordia was separated from the laterally adjacent tissues by longitudinal cuts on four sides. The cuts were 400 μ to 430 μ apart, and the result was a longitudinal central sector of shoot tissues topped by the shoot apex and its three youngest primordia. The whole split stem was then placed horizontally under the microscope and transverse cuts 400 μ to 430 μ apart were made. The uppermost piece (A) contained the shoot apex, three youngest foliar primordia and a small amount of basal maturing tissues; the middle piece (B), and the basal piece (C), were cubes of progressively maturing tissues (fig. 1, 2).

Within the limits of experimental error, A-, B-, and C-pieces were cut to the same volume (0.06 to 0.08 mm^3). Since the A-piece was measured longitudinally from the tip of the shoot apex down to the first transverse cut, the empty space around the dome of meristematic tissues represented a subtraction from the total volume of 400 μ^3 to 430 μ^3 that was achieved in the B- and C-pieces.

The small volume of these A-, B-, and C-pieces of tissue was arrived at not by attempting to determine the smallest purely vegetative mass of tissue from which a complete plant could be grown, but by dissecting out the shoot apex with as few as possible of the youngest primordia, for these arch over the dome of meristematic tissues. It was found very difficult to dissect out the shoot apex uninjured without these youngest foliar primordia.

White (1933) pointed out that the region of the shoot apex of *Stellaria media* is normally aseptic. Complete asepsis prevails around the shoot apices of *Tropaeolum* and of *Lupinus,* even though the former is in a loose bud that is open to the surrounding air. The meristem of *Lupinus* is covered by many overlapping, sheathing leaf bases that might protect it from contamination. These results disagree with those of Keener (1945) who described a "mycoflora" on the outside and inside of the buds of certain trees. A plausible theory to account for the normal asepsis of the embryonic region is that the constant movement by growth of the tissues away from the tip of the dome of meristematic tissue carries away any microorganisms that accidentally lodge there.

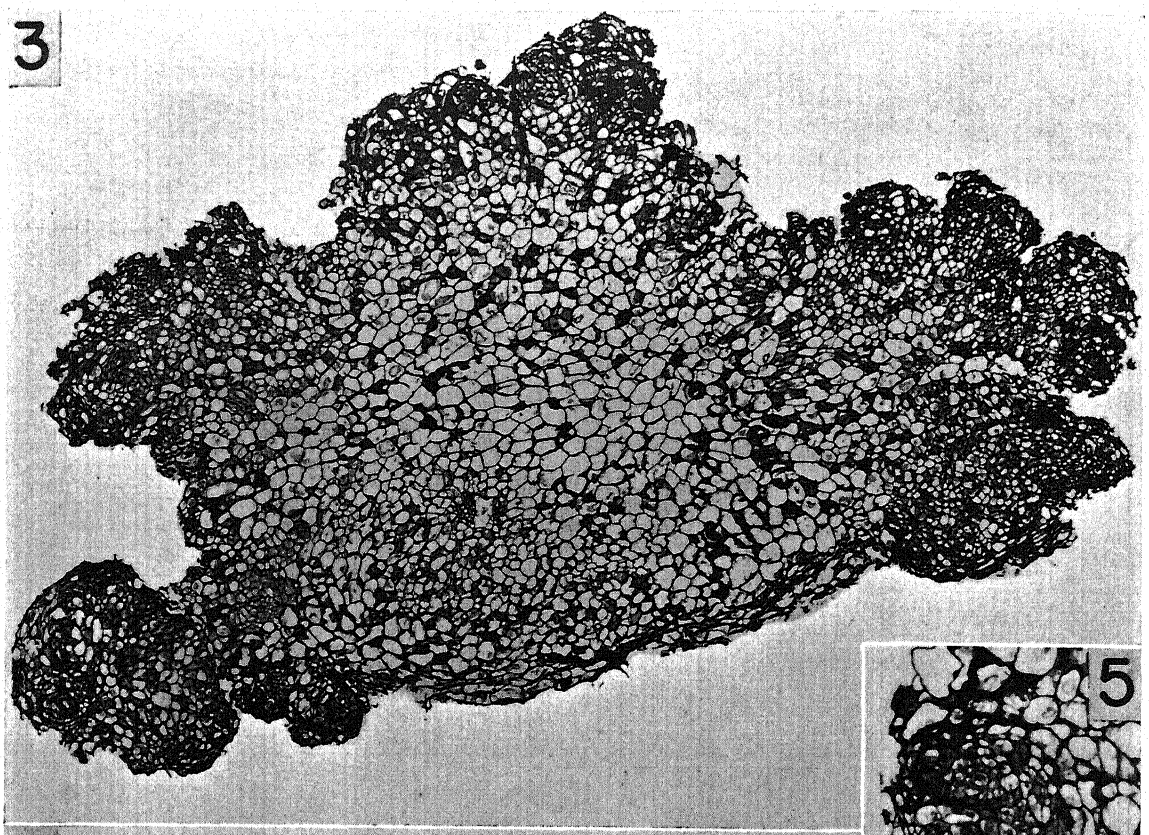
No provision was made to achieve or maintain asepsis. Inoculations were performed in the laboratory. The A-, B-, and C-pieces were excised with sterile knives and transferred directly to separate flasks or tubes of sterile media. The knives were sterilized by storing and washing them in absolute ethyl alcohol. Contaminations were always less than 3 per cent.

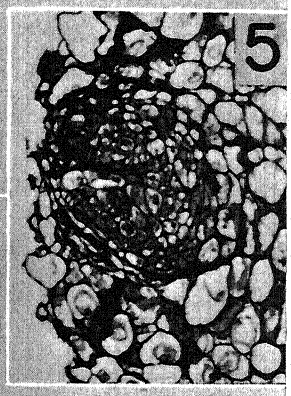
The medium used was usually the modification of Pfeffer's culture fluid listed by Robbins (1942)[2] plus certain microelements. Parallel cultures were usually made both with aqueous medium, and with 0.3 per cent agar.

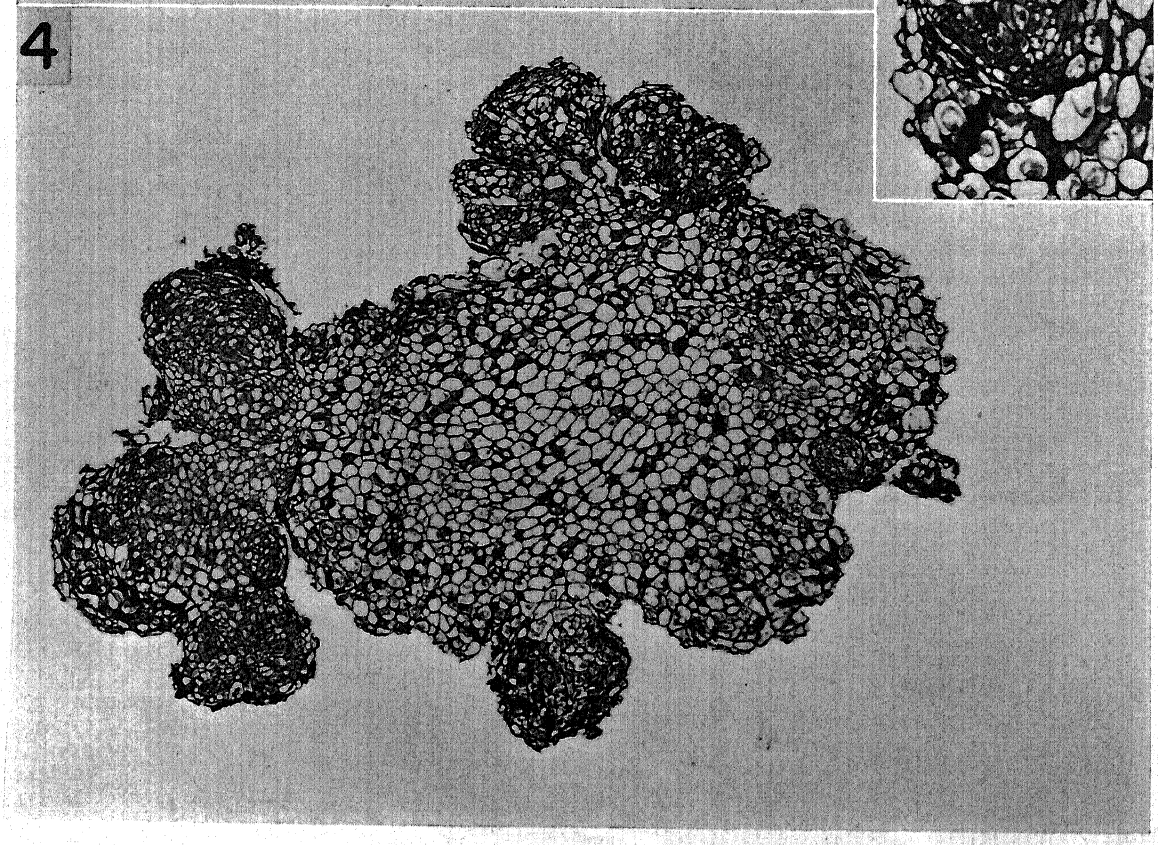
The agar used was washed daily with distilled water for thirty days. Each change of water remained over the agar for twenty-four hours. During

[2] Grams per liter of: $Ca(NO_3)_2$, 0.333; $MgSO_4 \cdot 7H_2O$, 0.083; KNO_3, 0.083; KCl, 0.042; KH_2PO_4, 0.083; sucrose, 20.0; and mg. per liter of: H_3BO_3, 0.06; $MnCl_2 \cdot 4H_2O$, 0.04; $ZnCl_2$, 0.15; $CuCl_2 \cdot 2H_2O$, 0.05; $Na_2MoO_4 \cdot 2H_2O$, 0.03; $FeCl_3 \cdot 6H_2O$, 0.24.

Fig. 1–2.—Fig. 1. Longisections of the shoot apex and subjacent regions (A, B and C) of *Tropaeolum majus* which were grown in sterile culture. These regions indicate areas of approximately 400 μ^2. The B- and C-regions are shown here bordered by provascular tissues, which, however, did not always occur in pieces excised from very stout, vigorous stems (193×).—Fig. 2. Longisections of the stem tip (A) and the subjacent regions (B and C) of *Lupinus albus* that were grown in sterile culture. These regions indicate areas of approximately 400 μ^2. Only the upper corners of the B-piece ever contained any provascular tissues (193×).


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this treatment, the agar changed color from brown to white.

Cultures of A-pieces in aqueous medium either floated or sank. Floating cultures were made by gently placing the A-piece with its cut base upon the surface of the medium; the tissues then sank up to the waxy surface of the foliar primordia and shoot apex. If the container were carefully handled, the A-piece would float for months. Submerged cultures were made by shaking the container during inoculation.

The B- and C-pieces always sank in the liquid medium. They would not float on the surface, apparently because all of their surfaces were fresh cuts.

The consistency of the agar medium was such that tissue pieces could be placed upon, or pushed beneath the surface.

Parallel cultures were carried out in the diffuse daylight of the laboratory, in diffuse daylight in a constant temperature room at 15°C., and in a dark room without controlled temperature. No attempts were made to subculture these tissues. They were usually allowed to grow as long as possible in the original medium. These experiments were, therefore, entirely concerned with the residual growth of tissues.

EXPERIMENTS WITH TROPAEOLUM.—*Results on aqueous medium.*—The A-pieces grew very slowly when floated on the top of the aqueous medium either with or without the accessory substances used.[3] Such cultures have lived either in dark or in light (where no green color developed) for as long as nine months without the formation of roots or leaves. The only growth was a slight increase in the basal tissue. Such old cultures occasionally produced roots, but the leaves remained small. The longevity of these tissues in culture is comparable to that achieved by La Rue (1938) who kept alive certain organs (petals, sepals, stamens, ovaries, etc.) in culture for as long as a year. The retention of some capacity for regeneration, i.e., production of adventitious roots, indicates that the ability of these stem tips to develop had not been entirely lost. Unautoclaved coconut milk added to the medium (9 per cent by volume) did not accelerate this slow growth.

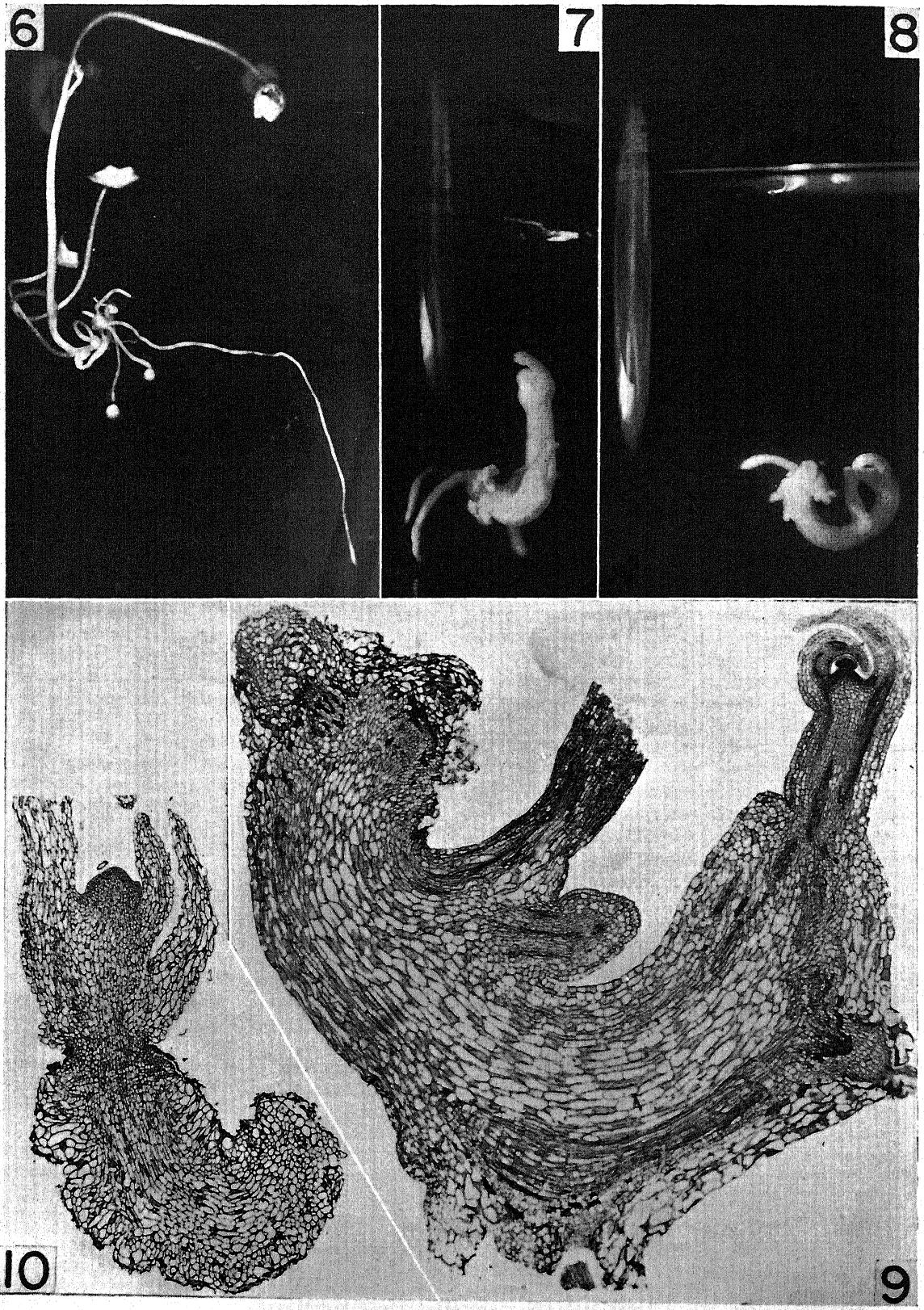
The A-piece grew little or not at all submerged in aqueous medium either with or without the accessory substances. Histological examination revealed that the cells of the shoot apex had enlarged, become highly vacuolate, and ceased to resemble the normal meristematic cells.

Neither the B- nor the C-pieces of the shoot grew when submerged in the aqueous medium, either with or without the accessory substances, and their cubical shapes were usually retained for months without any enlargement. Unautoclaved coconut milk added to the medium, however, permitted their vigorous growth. Milk from mature, unripe coconuts was the most effective. Nine per cent by volume was a suitable concentration in the Pfeffer's solution. The first indication of growth was the sticking of the cube to the bottom of the container. Growth was much more vigorous in the light than in the dark, but the manner of growth was essentially the same under both conditions. Neither group of cultures developed chlorophyll.

Histological examination revealed that single cells or small groups of cells near the cut surfaces underwent abundant mitoses and produced irregular masses of dividing cells (fig. 3, 4). Nests of rapidly dividing cells occasionally crushed the surrounding cells (fig. 3, 4, 5). These regions of dividing cells did not remain spherical, but enlarged and later formed a small-celled border of the callus. Although the inocula (see fig. 1, B and C) were frequently bordered on four sides by provascular tissue which showed various stages of maturation into vascular tissue, the growth in culture appeared to be uniform over the entire surfaces. The young pith cells grew as well as the provascular cells. Internal cells of the original pieces underwent some enlargement but apparently never divided. The resulting mass grew for three or four months at 15°C. and for shorter times at higher temperatures. The final volume was often one thousand or more times that of the inoculum. It was usually a mass of pure parenchyma; no cork-like tissue occurred on the surface and lignified cells in the interior were very rare. The lignified cells were always very short reticulate tracheal elements. They did not arise from cambium-like activity, but were derived haphazardly by maturation of already-present parenchyma cells or from newly-formed cells. No phloem was ever observed. The normal stem produces the normal variety of conducting elements: annular, spiral, scalariform, reticulate and circular-bordered pitted tracheal elements in addition to normal sieve tube elements and phloem fibers. It is difficult to interpret the restricted kind and amount of vascular tissue in these cultured calli. Have these partially differentiated tissues lost "capacity" for development, or do they merely fail to display it under the cultural conditions? A further restriction of development is the failure of the cultures to produce a cambium. The normal stem of *Tropaeolum* undergoes considerable secondary growth. In general,

[3] Concentration of accessory substances in mg. per liter of medium: thiamine hydrochlorine, 0.15; ascorbic acid, 20.0; nicotinic acid, 0.5; pyridoxine hydrochloride, 0.2; adenine sulfate, 0.2; succinic acid, 25.0; riboflavine, 0.1; calcium pantothenate, 0.5; p-aminobenzoic acid, 0.2; inositol, 0.2. Biotin, 4 gamma/liter.

Fig. 3–5.—Fig. 3. Section of the callus grown in culture in the light from a B-piece of *Tropaeolum*. Medium contained 9 per cent unautoclaved coconut milk from mature, unripe seeds. Marginal "meristematic" cells surround the inner, "non-meristematic" cells. 30×.—Fig. 4. Section of the callus grown from a C-piece of *Tropaeolum* under the same conditions as above. 30×.—Fig. 5. One of the spherical patches of "meristematic" cells during the early stages of its development. Crushed cell walls of adjacent cells surround this "meristematic" region. 76×.


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the only anatomical differentiation that these cultures usually showed, aside from the few tracheal elements, was that of the peripheral "meristematic" parenchyma and the internal "non-meristematic" parenchyma.

The masses of "meristematic cells" might be interpreted as incipient root or shoot primordia that are unable actually to produce true organs, or even recognizable embryonic stages of such organs. Howard (1940) suggested that root and shoot primordia in auxin-treated cabbage stems start as "generalized" meristems and only quite late take on the characteristic growth patterns of the organs. Since the young pith cells of the inocula were already partially "differentiated" or "matured," in comparison with the cells of the shoot apex, the formation of the actively dividing marginal "meristematic" tissues may be interpreted as a dedifferentiation.

Growth of the B-pieces was always more vigorous than that of the C-pieces. This decreasing capacity for growth and development along the axis may be correlated with the increasing size and decreasing number of cells, and the decreasing respiratory rate (Ball and Boell, 1944) in these cubes of tissue. The B-piece had ca. 16,000 cells and the C-piece ca. 8,000 cells in the one series of counts. Cubes of tissue taken below the level of the C-piece showed progressively decreasing growth of the type described down to the 2 mm. level. Cubes taken from below that level displayed no growth.

The growth of these cultures was essentially apolar or radial. The regions of "meristematic" cells grew over the surface of the culture without regard to the original polarity.

Growth in agar.—A-pieces floated base down on the surface of agar produced in many cases one or more adventitious roots. The shoot apex, exposed to the atmosphere above the surface of the agar, never produced normal leaves. If such a culture, after growth of one or more roots, was shaken so that it was submerged in the agar, the shoot apex soon produced several large, almost normal leaves that grew upward through the agar into the air.

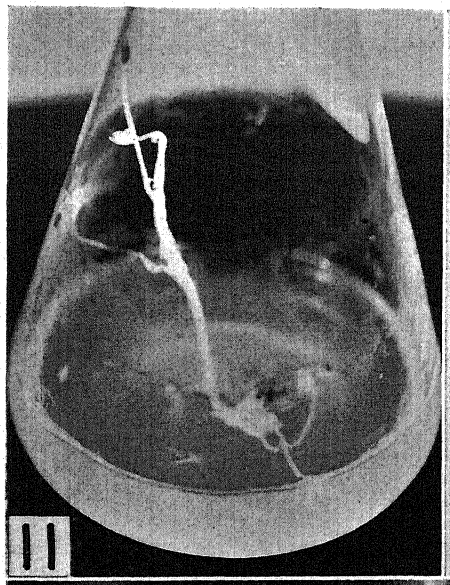
Agar cultures of originally submerged A-pieces grew in two different ways. In one type of development (in the light) the foliar primordia became intensely green. This behavior was quite different from that of the foliar primordia around the apex on the intact plant where these structures are not green. Later, these young leaves developed into mature peltate leaves that pushed through the agar and into the air. During the growth of these leaves, the basal tissue produced adventitious roots. Another type of behavior was as follows: in light the primordia did not become green, and the first sign of growth was the production a month or longer after inoculation, of adventitious roots by the basal tissue. During the next month, the primordia around the shoot apex produced leaves that grew into the air (fig. 12). The variation in manner of growth suggests that the presence of roots is not necessarily a prerequisite to the growth of the bud (cf. Went, 1938). De Ropp (1945) stated that excised stem tips of rye showed no meristematic activity unless, by some chance, a root was produced.

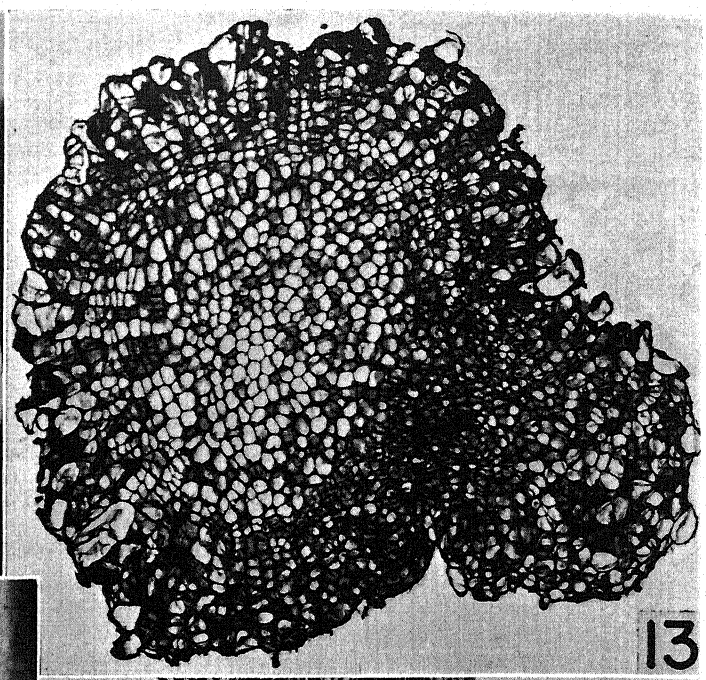
The first leaves produced by the shoot apices were very small and laminae were almost entirely lacking (fig. 7, 8 and 12 show some of them). These first leaves may be compared with those produced on isolated buds of *Ceratopteris thalictroides* that were grown on moist peat by Goebel (1908). He attributed the inhibition of size and abnormal simplicity of the leaves to a disturbance in nutrition which must be overcome before normal leaf formation can occur. Later leaves of *Tropaeolum* were larger, and finally, leaves on the sterile plants were normal in appearance, though much smaller than those on soil-grown plants (fig. 6). It is suggested that these buds of *Tropaeolum* had, in keeping with Goebel's suggestion, at first produced small, abnormally simple leaves before they had begun efficiently to utilize the sugar, salts, etc., in the culture medium; the later vigorous growth indicates that the bud utilized the culture medium to achieve almost normal growth. Many cultured plants attained heights of 8 to 10 cm. They were transplanted to soil where they grew normally. The growth of entire, normal plants from these tiny stem tips demonstrates that this region of the plant possesses the capacity to produce all tissues of the plant.

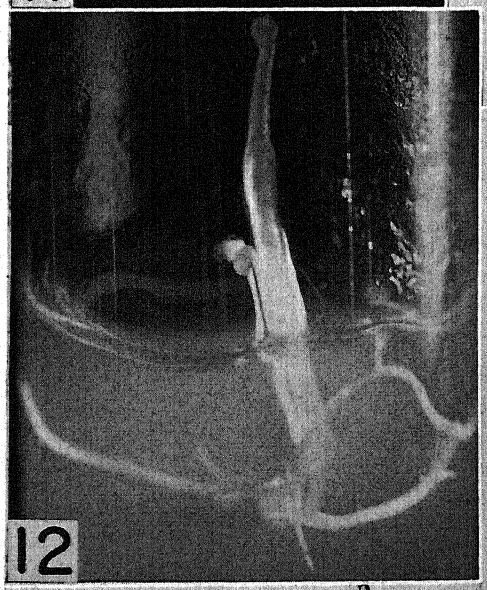
The percentage of A-pieces per series that grew into complete plants ranged from twenty down to five. Some of the probable causes of this variation are: inadequacies in the medium, inherent variations in the shoot apices, accidental injuries to the shoot apex, dormancy of shoot apices caused by cultural conditions.

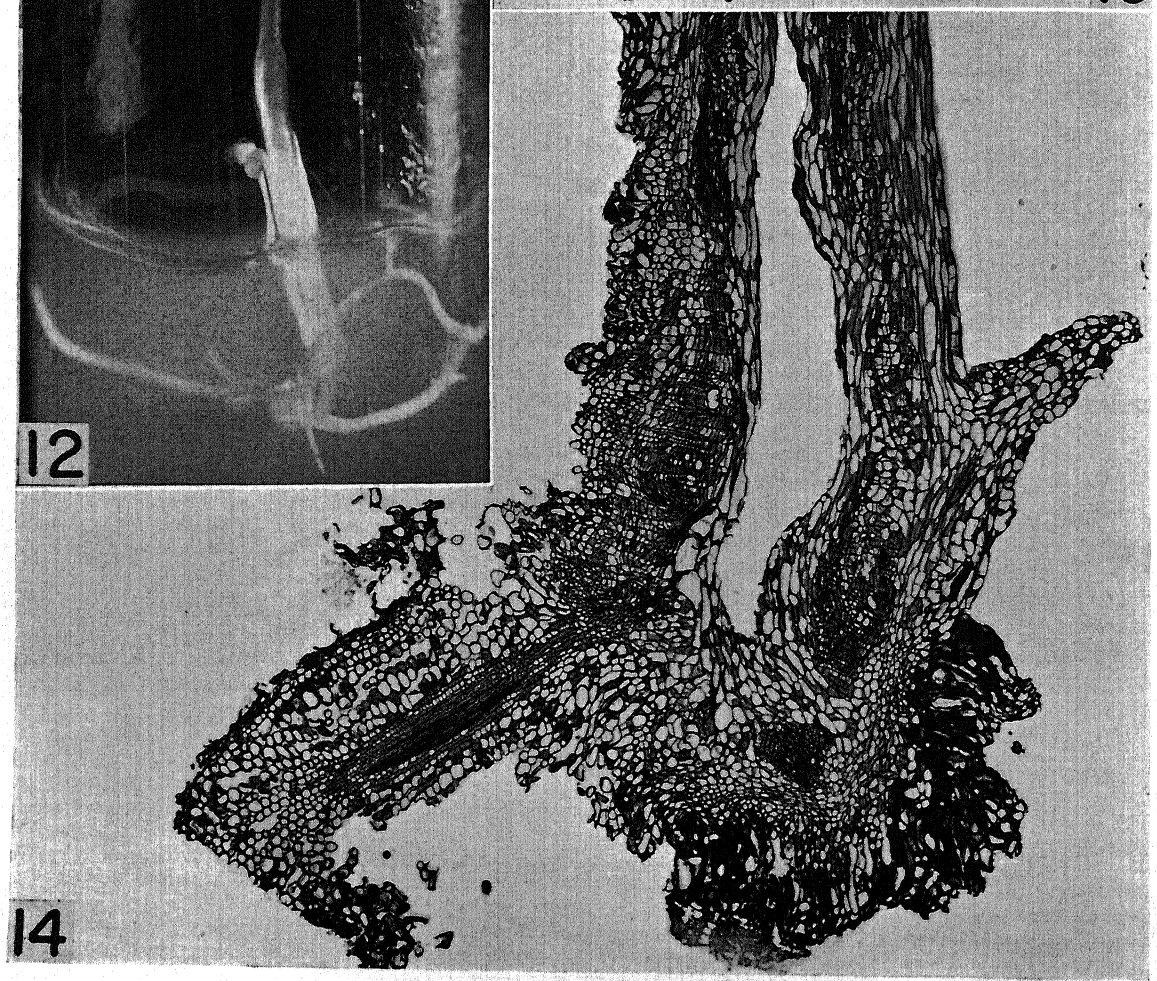
A-pieces were oriented, upon inoculation, so that the shoot apex would (1) point upward in the normal fashion, (2) point horizontally, and (3) point downward in the inverted position. These A-pieces, oriented in the three different positions, grew equal-

Fig. 6–10.—Fig. 6. Complete plant grown from a *Tropaeolum* A-piece (400 μ^3). 1×.—Fig. 7. Young, complete plant grown from a *Tropaeolum* A-piece (430 μ^3) which was oriented horizontally in agar. The subjacent stem curved so that the stem tip pointed upward normally. Basal callus bears several adventitious roots. Photo by Microtessar lens through wall of tube and agar. 3.4×.—Fig. 8. Young, complete plant grown from a *Tropaeolum* A-piece which was inverted in the agar. The subjacent stem curved so that the stem tip pointed upward normally. Bright horizontal line at top is the upper surface of the agar. 3.4×.—Fig. 9. Longisection of the basal part of a complete plant grown from a stem tip of *Tropaeolum* that had been oriented in the agar with the shoot apex pointing downward. The terminal shoot apex became dormant, and the leafy shoot that grew above the agar came from a basal adventitious bud. 30×.—Fig. 10. Stem tip of *Lupinus* that was inverted under the agar. The basal tissues curved so that if growth had continued, the stem might have been reoriented to point upward. 37×.


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ly well. In every case, whether the shoot apex had originally been pointing upward, horizontally (fig. 7), or downward (fig. 8), the new shoot produced by its activities finally became vertical with respect to gravity and its shoot apex pointed upward normally. The reorientation was caused by the curvature of the basal tissue (fig. 9). During the early stages of growth, the basal tissue became an elongated mass of stem-like tissue. This tissue curved in such a fashion that the shoot apex was shifted toward the normal orientation (if it did not already lie in such a position) and came to point away from gravity.

The curvature (negative geotropism) of the basal tissue of the A-piece, which served to bring the shoot apex back into proper orientation to gravity, was apparently controlled by the shoot apex. The polarity of the shoot apex was thus not changed by altering its position with respect to gravity.

The behavior of the basal tissues of the A-piece is also interesting histologically. The first-formed basal tissue had no epidermis, but its contact with the agar medium was the original cut surfaces (fig. 9). These cut surfaces obviously functioned to absorb water, sugar and salts from the medium. Basal tissues produced later had a more or less normal epidermis. The period of time during which the shoot apex produced basal tissue without an epidermis varied widely in different A-pieces. However, in all cases, a considerable time before the leaves started their rapid upward growth, the shoot apex produced basal tissue (stem) with an epidermis.

Little effect of the various accessory substances (footnote, p. 305) could be detected. In some cases, the adventitious roots seemed to be better developed than in cultures lacking these substances. Unautoclaved coconut milk was without benefit, but appeared to bring about abnormal growth; numerous adventitious buds arose from the much enlarged basal tissue. No one bud gained the ascendancy and consequently no normal plants were obtained.

While most A-pieces grew by activities of the original shoot apex, occasional stem tips produced adventitious shoot apices from basal tissue. Sometimes the adventitious bud grew while the original shoot apex did not (fig. 9); in other cases the reverse occurred, and in still others, both grew.

Production of adventitious buds and adventitious roots in the basal tissues demonstrates the regenerative capacity of the maturing tissues near the shoot apex. The B- and C-pieces never produced buds or roots, apparently indicating that these older, more differentiated tissues had no such capacity to regenerate. Supplementary experiments were carried out in which AB- and ABC-pieces (430 μ × 430 μ × 860 μ, and 430 μ × 430 μ × 1290 μ, respectively) were grown in agar. These long inocula grew less well than the A-pieces, and their basal tissues (corresponding to the B- or C-pieces) never produced adventitious roots or buds.

Growth of the A-piece beneath the surface of the agar demonstrates the validity of the measurements of its respiratory rate (Ball and Boell, 1944). The A-piece was found to consume less oxygen per unit time than the subjacent maturing tissues. The environment under the agar would be one with a low supply of oxygen.

The B- and C-pieces of the shoot, when inoculated into agar without coconut milk, grew for a month or longer and increased greatly in volume. Histological examination revealed that most, if not all, of their volume increase came from cell enlargement. Interesting evidences of maturation were found in these tissues after six weeks. Apparently the provascular, young cortical and pith tissues originally present underwent maturation to produce approximately their mature counterparts. The tissues never developed chlorophyll and never proliferated. They became brown after two or three months.

The B- and C-pieces grew in agar with coconut milk in the same fashion as, but less abundantly than, they did in liquid medium containing this liquid endosperm.

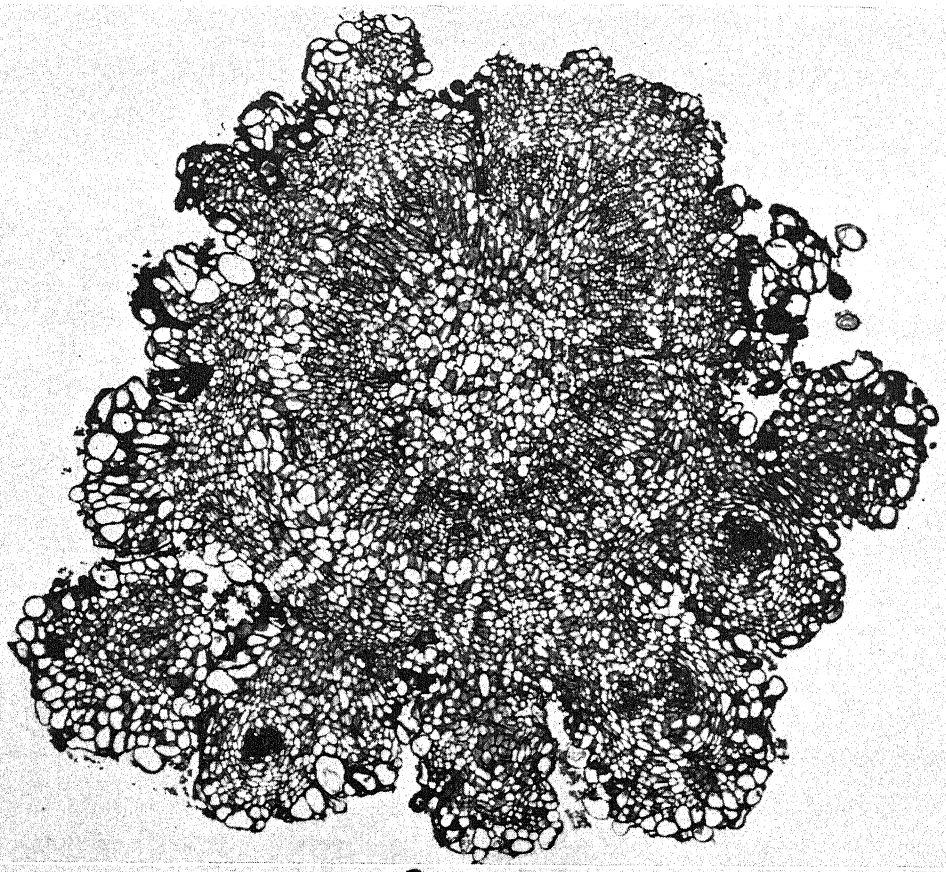
EXPERIMENTAL RESULTS WITH LUPINUS.—Since *Lupinus* and *Tropaeolum* differ physiologically and anatomically, it was not unexpected that their tissues should behave differently *in vitro*.

It had been determined earlier (Ball and Boell, 1944) that the A-piece of *Lupinus* exhibits the highest respiratory rate of the shoot; there is a gradient of decreasing oxygen consumption in the A-, B-, and C-pieces of tissue. Correlated with these data are the results of attempting to grow A-pieces beneath the agar. The bright green foliar primordia expanded but never reached the air above the medium. The growth rate decreased and finally stopped, and usually within a month, the culture turned black. It is obvious that there was insufficient oxygen to permit the growth of the A-piece.

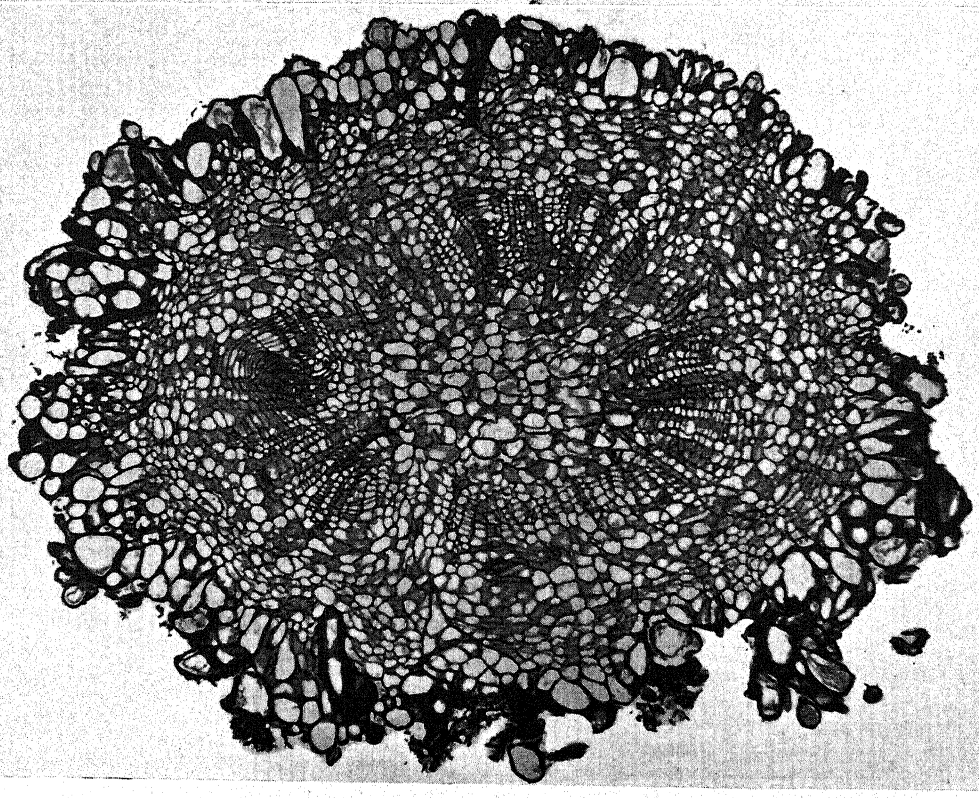
Various orientations of the A-piece with respect to gravity in the agar gave approximately the same results as those for *Tropaeolum*. During the short time that the A-piece grew in reversed or horizontal orientations, its basal tissues elongated and curved in a manner that would, if growth had continued longer, have brought the shoot apex to an upright,

Fig. 11–14.—Fig. 11. Culture flask containing a complete plant grown from a stem tip of *Lupinus* in the dark. 1×.—Fig. 12. Photograph with Microtessar lens of a tube containing a young, complete plant grown from an A-piece of *Tropaeolum*. The first leaves and terminal bud have just pushed through the surface of the agar. 2.6×.—Fig. 13. Section of a 2½-week-old culture of a B-piece of *Lupinus*. Origin of the spherical cambium is observed in the radial divisions of large parenchymatous cells at considerable distances beneath the original cut surface. 67×.—Fig. 14. Longisection of the basal part of a complete plant grown from an A-piece of *Lupinus*. The lower part is covered by elongated, collapsed cells, and an adventitious root is at lower left. Abundant cambial activity occurs in the upright stem, which is hollow. The stem for a considerable distance at the base does not possess an epidermis. 31×.

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vertical position (fig. 10). The polarity of the shoot apex of *Lupinus* is, therefore, not altered by changing its orientation to gravity.

Best growth with agar occurred in the dark when the A-pieces were not submerged but were carefully placed with cut surfaces downward on the medium. They grew for six months or longer and produced shoots 6 cm. or more in height (fig. 11). Adventitious roots were formed by the basal tissues only after four or five months when the shoots were usually 3–4 cm. long and had several leaves. Previous to the formation of roots, these shoots obviously obtained all their water, salts and sugar through the callus-like basal tissue (fig. 14). The basal tissue at first lacked an epidermis, but later it had a discrete epidermis. Abundant cambial activity occurred in very short cambial initial cells. Cambium-like tissue extended horizontally beneath the lower cut surface, which was covered by a mass of dead, tannin-filled cells. The stem early became hollow by break-down of central cells.

The leaves on such shoots remained very small, as is characteristic of etiolated plants, and the laminae did not unfold. The stems of these etiolated plants were many times as long as those of light-grown cultures.

The accessory substances (footnote, p. 305) had little effect on growth of the A-pieces. Unautoclaved coconut milk (9 per cent by volume) led to the growth of numerous adventitious buds, and no normal plantlet was obtained.

A-pieces submerged in aqueous medium usually grew in that position only for the short time necessary for the first leaf to expand. The matured tissue of this leaf contained sufficient gases to cause the culture to float near the surface.

A-pieces floating on the surface of the aqueous medium from the time of inoculation grew in light to produce several large, frequently abnormal compound leaves. The stems remained very short. In the dark, such floating cultures produced shoots 4 cm. or more in length after periods up to six months. No roots were ever produced in these aqueous cultures either in light or dark.

The B- and C-pieces grew neither in the aqueous medium nor in agar with or without the accessory growth substances. Within two weeks after inoculation, the tissues became dark brown or black. In the aqueous medium containing nine per cent unautoclaved coconut milk and in the light, they began to grow immediately. First manifestation of growth was a slight increase in volume, a loss of the cubical form, and an adherence to the bottom of the container. Both B- and C-pieces grew in light to produce large, green, radially differentiated structures (fig. 15, 16). These groups of tracheal elements suggested separate vascular bundles. The lignified tracheal elements were usually very short—almost isodiametric—and resembled closely in size and shape the surrounding parenchyma cells. The presence of but a single type of woody element in the xylem suggests a restriction of capacity for anatomical differentiation. The normal stem of *Lupinus* produces the usual types of xylem elements in the primary wood: annular, spiral, scalariform, reticulate and pitted. The cultural conditions apparently accelerated the process of cambium formation. In the normal stem, pronounced cambial activity occurs only in the older, lowermost parts of the stem. In the calli, a continuous sheath of cambium-like cells extended entirely around the zone of tracheal elements. Cambium cells arose by division of large, highly vacuolated pith cells (fig. 13). Apparently a cambium-like layer was formed beneath each cut (wound) surface in the manner postulated by Bertrand (1884) for the "free surfaces" of plant tissues. The activity of this spherical cambium led to a radial or apolar type of growth. The original polarity of the tissues was not retained. Presumably the majority of the vessel elements internal to the cambium arose by the activities of that meristem. It is possible, however, that some of them were merely parenchyma cells transformed into lignified elements in the manner described by Sinnott and Bloch (1944, 1945) for regenerated vascular strands in the pith of wounded *Coleus* stems.

Light had a pronounced effect upon the anatomy of these masses of tissue. Dark-grown cultures were yellowish-white and had the same tissues with the exception of the cambium-like layer. Cell divisions occurred, however, in a symmetrical manner so that a radially arranged mass was achieved.

The tracheal elements had no definite orientation; their long axes (if any) were in various directions. Outside the zone of tracheal elements, the cambial region produced tiers of parenchyma cells, on the outer limit of which were found occasionally what appeared to be sieve tube elements and their adjacent companion cells. These putative phloem elements usually occurred as single, short rows of sieve tubes. These cultures resemble anatomically those of carrot described by Gautheret (1938, 1940).

The differentiation and the secondary growth that the B- and C-pieces underwent are interesting because, at the time of inoculation, these tissues showed only the beginning of maturation into pith (fig. 2). Provascular tissue was found only occasionally in the B-piece and never in the C-piece.

The region external to the phloem in these cultures may be considered cortex. It consisted of large, thin-walled parenchyma cells. The entire callus was covered by several layers of large, thick-walled, brownish, empty cells. Histological exami-

Fig. 15–16.—Fig. 15. Section of a two-month-old culture of a B-piece of *Lupinus*. The spherical cambium displays considerable activity. Around the margins of the original callus, numerous spherical proliferations, each with a central core of tracheal elements, are observed. 50×.—Fig. 16. Section of a two-month-old culture of a C-piece of *Lupinus*. Considerable activity has occurred in the spherical cambium. This callus differs from that of the B-piece in the lesser growth and the rarer occurrence of marginal spherical proliferations. 60×.

nation did not reveal the cause of their sticking to the bottom of the container.

A cambium-like region beneath the brownish surface cells occasionally produced small spherical proliferations that remained attached to the original callus. Each of the proliferations had a core of tracheal elements (fig. 15), which was sometimes attached to a radially oriented strand of elongated parenchyma cells that was continuous with the vessel elements of the original callus. In a primitive sense, these proliferations may be considered lateral appendages and probably represent the beginnings of vegetative reproduction of the callus. Gautheret (1940) described similar proliferations upon cultured slices of carrot.

Growth of the B-pieces was usually more vigorous and prolonged than that of the C-pieces. Attempts to grow pieces of tissue of equivalent volume subjacent to the C-pieces were unsuccessful. Cultures of successive 1 mm^3 cubes of tissue down the stem were also attempted. Growth occurred only in the uppermost such piece below the stem tip (assumed to be 430 μ^3). Capacity for development here appears to be less than it is for comparable regions of *Tropaeolum*.

Attempts to grow AB- and ABC-pieces (430 $\mu \times$ 430 $\mu \times$ 860 μ, and 430 $\mu \times$ 430 $\mu \times$ 1290 μ, respectively) were not so successful as the cultures of the A-pieces alone. No adventitious roots were produced by the basal tissues of these long inocula.

The B- and C-pieces appear, under the cultural conditions, to be unable to develop organs, while they have retained the ability to undergo cell division.

The decreasing capacity for growth and development of these cubes of tissue along the axis may be correlated with their decreasing respiratory rate (Ball and Boell, 1944) and with their increasing size and decreasing number of cells. The B-piece had approximately 13,500 cells, while the C-piece had only approximately 5,000 cells.

Discussion.—The callus-like proliferation on the bases of the A-pieces may be an indication that absorption of water, sugar and minerals occurred over the entire cut surface. It suggests that the substances absorbed are transported through all of the cells upward to the shoot apex, instead of being transported exclusively through the provascular tissues. From the water, mineral salts and sugar absorbed by these tiny stem tips, the shoot apices were able to grow and produce approximately normal shoots. This result contradicts the interpretation of de Ropp (1945) that the stem tip is not physiologically adapted to absorb already elaborated nutrients direct from an external medium. The earlier works of La Rue (1936) and Loo (1945) also agree with the present writer's results.

Tissues of the A-piece were relatively little affected by isolation from the rest of the plant. The great developmental capacity of the shoot apex is not directly related to the dominance of the terminal shoot apex over lateral shoot apices. The A-pieces of both *Lupinus* and *Tropaeolum* produced complete plants *in vitro* although their shoot apices are dominant, and not dominant, respectively, over the lateral apices. In fact, the A-pieces of *Tropaeolum* grew better than those of *Lupinus*, and produced plants of such vigor that they could grow normally in soil.

The great capacity for development of the shoot apices is not directly related to the rate of respiration exhibited by the A-pieces (see Ball and Boell, 1944). The rate of oxygen consumption of the A-piece of *Lupinus* is the highest of the shoot, and it produced entire plants in culture only after six months in the dark. The rate of oxygen consumption of the A-piece of *Tropaeolum* is less than that of the immediately subjacent tissues, yet it produced entire plants in culture after a month in the light. Correlated with its low oxygen consumption is the fact that the A-piece of *Tropaeolum* grew best and produced entire plants only under the agar. Such growth in a low-oxygen environment suggests that this shoot apex on the intact plant normally grows under similar low-oxygen conditions. It is difficult, however, to understand how the shoot apex preserves such a condition when it is freely exposed to the atmosphere. Further complicating facts are that the A-pieces floated on agar or aqueous medium produced no leaves or stems, but occasionally only roots; when they were submerged in aqueous medium, they did not grow at all.

Merry (1942) found that certain grass embryos grew best in culture under reduced oxygen supply. Kakesita (1933) concluded that the warm-bath treatment of buds caused the formation of products of anaerobic respiration that stimulated their sprouting. Ruhland and Ramshorn (1938) suggested that plant meristems are characterized by aerobic fermentation and therefore low oxygen consumption. Prevot (1939) attrbiuted bud formation in *Begonia*-leaf segments to anaerobic conditions. White (1939b) suggested that a difference in oxygen supply was the cause of "differentiation" (formation of vegetative buds and roots) on tobacco callus grown in sterile culture. Callus beneath liquid or agar produced buds, while that on the surface remained without buds. Boresch (1924, 1926, 1928) suggested that the warm bath causes anaerobic respiration in buds and leaves and forms products that stimulate the growth of cells. Reducing the oxygen supply does not appear to be the sole environmental factor responsible for the growth of the A-pieces of *Tropaeolum*. Two factors seem to be operative: submergence of the A-piece and the presence of agar in the medium. The agar, although thoroughly washed, may have contained certain substances helpful to the growth of these tissues.

The requirements of the stem tips of *Lupinus* for oxygen were such that they would not grow submerged, but only on the top of the agar or aqueous medium. This behavior suggests that their respiration is entirely aerobic and that the theories

of Kakesita, Ruhland and Ramshorn, Prevot, White, Boresch do not apply to all buds.

The B-piece of *Tropaeolum,* although it possesses a higher respiratory rate than the stem tip above it, did not produce an entire plant, but only a callus. The C-pieces of both *Tropaeolum* and *Lupinus* display lower respiratory rates than the respective B-pieces, and there is a correlation here in that the C-pieces showed less growth than the B-pieces. The increased "maturation" or "differentiation" (increased vacuolation, increased intercellular spaces, increased cell size, decreased cell number, etc.) of the C-pieces is also correlated with their lesser growth.

The supplementary series of cultures below the C-pieces gave results which suggest that the capacity for growth in these maturing pith tissues is gradually lost with increasing distances below the shoot apex. The lower limits that would grow in culture were approximately 2 mm. for *Tropaeolum* and 1 mm. for *Lupinus* below the buds. It appears that the pith of *Lupinus* matures at a faster rate and loses its capacity for development sooner than that of *Tropaeolum.* This difference may be correlated with the fact that the pith of *Lupinus* breaks down, leaving the mature stem hollow, while that of *Tropaeolum* usually remains in the living condition.

When long pieces (1 mm. $\times$ 1 mm. $\times$ 10 mm. long) below the stem tip (assumed to be 430 μ^3) of *Tropaeolum* were grown, callus formed in the tissues immediately subjacent to the stem tip and occasionally produced adventitious buds in a position corresponding to that of the B-piece. No roots were formed. The callus decreased in intensity in the subjacent regions and did not occur at all 7 to 10 mm. below the stem tip. These results indicate that if a sufficient mass of inoculum is attained, regeneration may occur where it would not come about in very small inocula. It can be assumed that some contribution was made by the large mass of tissue to the regeneration. The possibility must be kept open that if the B- and C-pieces had attained sufficient size in culture, their marginal meristems might have produced buds and shoots. Similar pieces (1 $\times$ 1 $\times$ 10 mm.) of *Lupinus* stems displayed callus formation at the upper ends, but formed no buds.

The A-pieces of both *Tropaeolum* and *Lupinus,* when they lived and grew in sterile culture, always preserved their normal type of growth; their shoot apices continued to function as embryonic regions that produced tissues differentiating into parts of the shoot (leaves and stem) plus adventitious roots. The shoot apex never became transformed, even in the presence of unautoclaved coconut milk, into callus. When it grew, it always produced an approximately normal shoot. Such behavior agrees with the hypothesis adduced by Child (1915, 1929) which stated that the embryonic region of the shoot tip of higher plants is an independent, self-determining region of the plant, that the apical region is capable of developing, at least to an advanced stage, in the complete absence of the other parts of the body. He further suggested that the growing tip is a fundamental factor in the unity and order of the plant individual, and that in relation to other growing tips and to environmental conditions, it determines the general form and pattern of organization of the plant. Thoday (1939) suggested that the shoot apex is a self-determining and dominant center of development, that it controls the polarity of the shoot, and that the apical bud as a whole controls primary development and secondary growth in the stem below. Church (1920) suggested that growth impulses radiated from the center of the shoot apex and that the apical meristem therefore controlled the growth and differentiation of the subjacent tissues, particularly the phyllotaxis and vascular system. Wardlaw (1944) presented a similar hypothesis in his work on the development of the vascular system of certain pteridophytes. He postulated that the initial differentiation of vascular system will always be observable immediately underneath an actively growing apical meristem and in the path of substances diffusing from it. He considered, therefore, that the shoot apex controls the differentiation of the subjacent tissues.

Approximately the opposite point of view has been presented by several other authors. Lestiboudois (1840), Priestley, Scott and Gillett (1935), Smith (1941), Esau (1942), Crafts (1943a and b), Majumdar (1942), Sterling (1945) all suggested from studies of the developing vascular system and of phyllotaxis that the shoot apex is not a controlling center of shoot development, but merely a plastic region that operates under the control of stimuli that are sent to it from the mature subjacent tissues.

While it is very probable that the rôle of the shoot apex in the growth of the higher plant will be found to lie somewhere between these two opposite points of view, the behavior in culture of the stem tips recorded by the present author may be considered experimental evidence of the independent nature of the shoot apex and that capacity to develop the entire plant resides in the small A-pieces. These results indicate that the "guiding" effect of older, subjacent tissues upon the meristematic ones does not necessarily occur.

The A-pieces had no vascular tissue except possibly some young primary phloem developing in the provascular strands in and beneath the three foliar primordia. Since these three original foliar primordia attained only very small size, it can be assumed tentatively that their influence was negligible on the development of the vascular system of the plant *in vitro.* The plants produced by the growth of these tiny buds in culture displayed normal vascular systems that only differed from those of soil-raised plants by their reduced size. The shoot apices of the A-pieces, separated from the mature, subjacent vascular systems and other tissues of the parent plants are thus demonstrated to be capable of determining the production and dif-

ferentiation of the entire subjacent plant body. Galligar (1939) demonstrated that 1 mm.-long excised root tips of sunflower, without demonstrable vascular tissues, could show enormous growth in culture and produce normal roots with vascular systems.

The B- and C-pieces of tissue, under the conditions employed, seemed to possess less potential for development. These tissues of *Tropaeolum* enlarged in agar without coconut milk and showed evidences of maturation, but no cell divisions. In aqueous medium with coconut milk they formed calli with irregular masses of rapidly dividing cells around the margins of non-dividing parenchymatous cells. The B- and C-pieces of *Lupinus* showed equally small potentialities for growth *in vitro*. They would grow only in aqueous medium with coconut milk, where they produced radially differentiated calli that did not resemble the stem of the normal plant but rather certain growths that sometimes occur after wounding. A spherical cambium produced xylem centripetally and putative phloem centrifugally. The vascular tissue was abnormal in cell shape and was without consistent orientation.

The B- and C- pieces of both *Tropaeolum* and *Lupinus* regenerated neither buds nor roots in culture. An indication that this small capacity for regeneration was inherent and not merely conditioned by isolation and smallness of inoculum was had in the results of growing pieces equivalent to the AB- (430 μ × 430 μ × 860 μ) and the ABC- (430 μ × 430 μ × 1290 μ long) pieces (cf. fig. 1, 2). The presence of the shoot apex on the ends of these pieces did not influence the basal tissues (the usual B- or C-pieces) to regenerate roots. The writer does not consider these experiments conclusive, for the buds grew much more poorly than those of the A-pieces alone. It is, however, possible that the growth of these buds was inhibited by the basal maturing tissues. Galligar (1939) discovered that 1 mm.-long excised sunflower root tips grew better than 10 mm.-long ones and suggested that the mature tissues inhibited the growth of the meristematic tip.

The persistence of the original polarity in the A-pieces of these plants in culture gave at least partial support to the interpretation of Vöchting (1906) that polarity of plant organs is irreversible. Furthermore, it was demonstrated in the present experiments that the shoot apex of the A-piece apparently controls the geotropism (negative curvature) of the subjacent stem tissue, causing it to curve in such a fashion that the stem tip became reoriented away from gravity. Rawitscher (1937), in his review, recalled the general conclusion that the apex of the stem and root is sensitive to the stimulus of gravity, while the curvature develops either exclusively or most conspicuously in the basal portion. La Rue (1936) encountered no change in the polarity of any of the buds that he grew in sterile culture. Bloch (1943) concluded that "the organized structures obtained from these (growing points) usually do not show any basic alterations of polar growth processes and polarity systems." The suggestions of Klebs (1904) and Pfeffer (1903) that the cells of the apical meristem have no inherent or fixed polarity are not confirmed. Vöchting (1885) carried out the very interesting experiment of raising a plant of *Papaver Rhoeas* on a horizontally oriented klinostat through the development of flowers, pollination, fertilization, embryo-formation and seed maturation. From time to time the seed development was investigated and found to be progressing normally. The next spring these klinostat-raised seeds were planted and normal plants grew from them. Vöchting's work, therefore, demonstrated that gravity changes did not affect the polarity of the shoot apex during vegetative growth and flower formation, and in the embryo during its development. Schüepp (1927) decided that the cells of the shoot apex are not polarized, since the mantel layers bend together and hit against each other, contradicting the nature of polarity. He, however, did not think of the possibility that the shoot apex as a whole is polarized, and that the individual cells work together in that respect, but thought that polarity should first occur in young internodes. The results of the present author do not support Schüepp's ideas.

The results of the present author are in agreement with comparable work on excised, sterile-cultured roots by Fiedler (1936). He demonstrated that response to gravity by growth curvatures is brought about in older tissues back of the embryonic apex. That the root apex controls such growth curvatures was demonstrated by removing the tip. Such decapitated roots did not, for a considerable time, respond by curvatures to changed orientation. There was later a physiological regeneration of the root tip and a normal curvature resulted.

The sudden and complete loss of the original polarity in the tissues of the B- and C-pieces of both *Tropaeolum* and *Lupinus* when grown in culture is to be strongly contrasted to the behavior of the A-pieces. This behavior contradicts Vöchting's (1906) assertion that the polarity of plant organs is irreversible. The subjacent, differentiating tissues of *Tropaeolum* in culture produced irregular areas of marginal "meristematic" cells that surrounded the internal, "non-meristematic" cells. The fact that coconut milk in the medium brought about growth with the loss of original polarity demonstrated the plastic nature of this polarity. It is important to note that coconut milk in the agar medium did not change the polarity of the A-pieces; its only effect was to bring about the growth of numerous adventitious buds and the production of several shoots per culture. The B- and C-pieces of *Lupinus* grew in a strikingly different manner in the medium containing coconut milk, but the original polarity here, too, was lost and the masses of tissue were apolar or radially differentiated. Similar loss of polarity in cultures of differentiating

tissues of carrot has been described by Nobécourt (1938).

It is suggested by the writer that these subjacent differentiating tissues have a polarity that is much more easily influenced by the environment than is that of the tissues of the A-pieces. Lacking a better interpretation, the author has further interpreted this plasticity of polarity in the differentiating tissues as a loss of the independent, self-determining nature so strikingly evident in the tissues of the stem tips. It is at the level of these youngest differentiating or maturing tissues in the shoot that the influence of the environment can make itself evident in changing the manner of growth in the absence of formative influences of the shoot apex.

The B- and C-pieces of tissue displayed restriction in the production of cell types. The best example of this is the xylem. White (1939a) noted a restriction of cell types in the calli of tobacco stem tissues. Only two types of mature cells occurred: the pith cell and the scalariform cell. The lignified xylem in cultures of the maturing pith of both *Tropaeolum* and *Lupinus* consisted of reticulate tracheal elements. Primary xylem of the normal stem has several types of lignified elements. A loss of potential for differentiation of all the normal types of xylem cells might be assumed to have occurred in these tissues during their maturation in the shoot. A second possible explanation is that the processes of excision and growth in culture may have brought about sufficient changes in the environments of these tissues to allow the peculiar type of radial growth and the restricted type of xylem to develop.

Much less subject to these environmental influences is the bud, particularly the embryonic tissues of the shoot apex. This independent, self-determining region of the higher plant (in the two examples tested) was not affected significantly by the processes of excision and growth in culture, but grew approximately normally and produced stems, leaves and adventitious roots. The bud of higher plants may be regarded as the seat of expression of genetic potentialities for vegetative development. These genetic potentialities are little affected by the normal environment, which attests to the independent nature of the bud. The subjacent, maturing tissues to which only a limited capacity for development appears to have come, on the other hand, are properly regarded as very susceptible to environmental influences during the developmental stages.

The techniques employed in this work—the excision of very small (0.06 mm^3 to 0.08 mm^3) pieces of tissue along the axis of the developing vegetative shoot, and their growth in sterile culture isolated from the influences of the entire plant—yield evidence of the great physiological differences that exist between the shoot apex and the older, maturing subjacent tissues. Furthermore, these techniques have yielded evidence that these great physiological differences come about in a very short space, probably less than 100 μ in the cells immediately subjacent to the shoot apex. In rapidly growing shoots, they undoubtedly come about in a very short interval of time—probably in a few hours. These differences may be labeled physiological differentiation. Comparable data for the root were presented by Robbins, Bartley and White (1936) who grew fragments of corn and pea roots in culture. They found that the cells at the tip of the embryonic region do not have fixed potentialities of development, as do the older cells of the plerome, but are capable of developing into any of the various elements of the root. They suggested that the determination (or loss of capacity) of tissues appeared to occur within 0.2 or 0.3 mm. of the apex of the root meristem.

Goebel (1902, p. 486) suggested that theoretically any somatic cell is capable of developing a new plant; that it does not do so is due to the conditioning of other cells around it. If this is neutralized (perhaps by separation or in other ways), the cell can again become embryonic if it is capable of existence in the isolated condition. Priestley and Swingle (1929) stated that potentially any cell is capable of meristematic growth and may produce any cell organization characteristic of the species, but that practically, its possibilities are definitely limited by its position in a complex organization. If these theories have any truth in them, then the present work demonstrates that the same environmental conditions will not enable equally small volumes of embryonic tissues and maturing tissues to develop in the same manner. According to these theories, it is necessary to bring about the proper conditions in order for differentiated vegetative tissues to display their full potentiality for development. It seems to this writer that these theories admit, in themselves, their own error, namely, that *different* conditions *are* necessary for the development of embryonic potentialities in differentiated tissues from those which are necessary for the growth of embryonic tissues. If such different conditions are necessary for the growth of these two types of tissues, then the essential differences of the tissues is established beyond question.

The writer freely admits that the cultural conditions in which the A-, B- and C- (and further subjacent) tissues grew were not necessarily an approach to the normal ones. The purpose was primarily to find conditions in which these tiny cubes of tissue would grow. It was certainly evident that the cultural conditions in which the A-pieces (containing the shoot apex) grew would not permit the growth of the B- and C-pieces, and vice versa.

The strikingly different types of growth displayed by the B- and C-pieces of *Tropaeolum* on the one hand, and of *Lupinus* on the other, argue against the possibility that coconut milk constituted a causative agent in that growth. This liquid endosperm appears merely to have brought certain organic substances to the culture medium, without which these small bits of tissue would not have displayed cell division and growth. Its utilization here

may be compared to that in the growth of small *Datura* embryos in *vitro* by van Overbeek, *et al.* (1942).

Linsbauer (1916, 1925) classified the meristems of higher plants according to their capacity for development. Only the cells of the shoot apex, the archimeristem, were considered totipotent, and the other subjacent meristematic cells had less power for development. Linsbauer thus considered that not every plant cell was totipotent, but that many of the less potent meristematic cells could regress or dedifferentiate and regain the totipotent condition of archimeristem.

Miehe (1926) suggested the extreme viewpoint that all living plant cells are not totipotent, but that they may be segregated into archenchyme (apical meristems, provascular and cambial tissues), and ergoplasts, or work cells. This segregation was assumed to occur at cell division and to be determined by the cytoplasm of the cells. The former term designated those cells capable of producing all types of cells characteristic of the plant; the latter comprised those cells that had lost unlimited potentiality, i.e., non-reproductive cells such as assimilatory cells, which do not have the mechanism for development.

Allen (1923, 1942) suggested that cytoplasmic differences between the cells of different tissues or organs may account for their inability to regenerate certain structures or organs while they are still alive and able to divide.

Goebel (1928, p. 56) stated that both extremes occur, and likewise intermediate cases.

The results described by the present author support the idea that there is a loss of potentiality in tissues below the apical meristem. However, our almost complete lack of knowledge of the processes of differentiation makes it necessary to be cautious in interpreting these experiments. It is entirely possible that small masses of maturing tissues such as those used in these studies are indeed lacking in capacity for development, as their growth indicated; but if these tissues could be grown to ten times the size attained, it is conceivable that there might be some contribution from the large mass of tissue to the regeneration of buds and roots. The formation of buds in the region of the B-piece of *Tropaeolum* when large ($1 \times 1 \times 10$ mm.) pieces were cultured may be considered evidence of this possibility.

The techniques of these experiments have merely served to demonstrate the lack of capacity of very small masses of maturing tissues when separated from the plant. Pursuing this line of thought, one might conceive of plant differentiation as a mere attenuation of the potentialities for development: regeneration, formation of organs, formation of cell types. The embryonic regions of the higher plant: the shoot apex, and to a much lesser degree, the root apex, have these potentialities to the highest possible degree in the smallest possible volume of tissues. Differentiating or maturing plant tissues have these potentialities in very reduced quantities, but as evidenced in the occurrence of regenerative phenomena in all types of mature living cells of the plant kingdom, these capacities may be concentrated at certain small regions in large masses of mature tissues.

SUMMARY

Very small stem tips (A-pieces) and cubes of subjacent maturing tissues (B- and C-pieces) having volumes of 400 μ^3 to 430 μ^3 of *Tropaeolum* and *Lupinus*, plants of different physiologic types, were grown in sterile culture with the view of obtaining data upon (a) capacity of embryonic vs. maturing tissues for development and regeneration, (b) relationship between capacity for development and respiratory rate, (c) polarity of the embryonic vs. the maturing tissues, (d) independence of stem tip (or shoot apex) from the rest of the plant, (e) production of subjacent tissues by the shoot apex, and (f) the theory of "totipotentiality" of all living plant cells.

The A-pieces utilized the water (in 0.3 per cent agar), sucrose and mineral salts of the nutrient solutions and demonstrated their pronounced capacity for development by growing into entire plants. The basal tissues of these plantlets always regenerated roots and occasionally adventitious buds. The stem tip of *Tropaeolum*, having a lower respiratory rate than its subjacent tissues, would grow into a complete plant only by culture beneath the agar. The stem tip of *Lupinus*, having the highest respiratory rate of the shoot, would grow into a complete plant only upon the surface of the agar. This behavior indicates that primary meristems cannot all be characterized by low oxygen consumption.

The polarity of the stem tips was not altered by inverted or horizontal orientations to gravity. Furthermore, the growth behavior (curvatures) in various orientations suggested that the shoot apex controlled the curvature of the subjacent tissues.

Plants grown from the shoot tips contained normal, although small vascular systems, which had been "determined" by the activities of their shoot apices entirely separated from the influences of the original mature plant body. Such development demonstrated the independent, self-determining nature of the apical meristem, and suggested that there is a very minor influence of the mature, subjacent plant body upon these activities of the shoot apex. According to this interpretation, the shoot apex is not merely a plastic region that obeys the impulses sent to it from the mature, subjacent regions of the plant, but actually controls or "determines" the growth and behavior of the subjacent regions.

The tissues subjacent to the stem tip (B- and C-pieces) grew best in aqueous medium to which unautoclaved coconut milk had been added. Those of *Lupinus* produced spherical masses that usually grew by cambium-like zones considerably beneath the original cut surfaces. Internally, this cambium produced parenchyma and very short tracheal ele-

ments; externally, it produced parenchyma and and some cells that were apparently sieve-tube elements. In contrast, the subjacent tissues of *Tropaeolum* had various regions of superficial cells that underwent rapid mitoses. The end result was an irregular mass of parenchymatous cells that only infrequently contained groups of tracheal elements. The calli from neither plant regenerated roots or buds. The original polarity of these subjacent regions was not retained in culture; the calli were radially differentiated and therefore without polarity. The growth of the B-pieces was always greater than that of the C-pieces; this result corresponded with the earlier findings that the respiratory rate of the B-piece was greater than that of the C-piece.

The results of these experiments have been interpreted to suggest that there is a decreasing capacity for growth and development in these small volumes of tissues down the plant axis. This phenomenon is labeled physiological differentiation. It is considered to agree with the suggestions in the literature that not all living plant cells are possessed of "unlimited capacity for development," but that the capacity for developing all parts of the plant is restricted to a very few tissues. The shoot apex is considered, in view of this experimental evidence, to possess in the highest degree the capacity for development of the entire plant. Tissues subjacent to the shoot apex (e.g., the B- and C-pieces) are considered to possess these capacities to only a limited extent. This interpretation is contrasted to other suggestions in the literature that theoretically every living plant cell is capable of producing any cell organization characteristic of the species.

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STREAMING MOVEMENTS OF SOLUTIONS IN PLANTS [1]

D. T. MacDougal

THE HYDROSTATIC SYSTEM.—The proposal by Dixon and Joly (1894) that the movements of solutions from the absorbing surfaces of roots to the transpiratory surfaces of leaves are due to differences in vapor pressure and that the strands of water in the conduits are not broken while being lifted to the tops of tall trees is known as the "Cohesion Theory of the Ascent of Sap." Earlier contentions as to the inadequacy of this thesis have been met by increasing knowledge of the cohesive properties of liquids and of the pattern of the hydrostatic system in plant tissues.

Demonstration that tensions in non-living conduits tended to draw their walls together and measurements of the resulting collapse are now taken as decisive evidence of the validity of the cohesion theory of the ascent of sap (MacDougal, 1921; Bode, 1923) (fig. 1).

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In the present paper the hydrostatic system is considered as constituting a continuous meshwork of liquid filaments in non-living conduits connecting through moist walls with all or nearly all of the highly hydrated or living cells of the plant. Tensions set up in any part of the system, notably in leaves, result in free flow in conduits and some withdrawals from living cells.

The greater part of the upward movement of sap in trees is in recently formed layers of wood; gums, tyloses, etc., obstruct the flow in older tracheids and vessels with a resulting lessened flow. In some trees these obstructions occur in only parts of the layers. In any case the liquids in the wood are under tension and constitute a reserve which may be drawn upon when tensions are increased, as would result under drought conditions. Such a depletion may empty some elements of the wood, which are then occupied by gases of the pneumatic system. Abrupt reduction of the transpirational pull may be followed by reentrance of solutions into woody elements by capillary action, an occurrence connected with autumnal leaf-fall in deciduous trees.

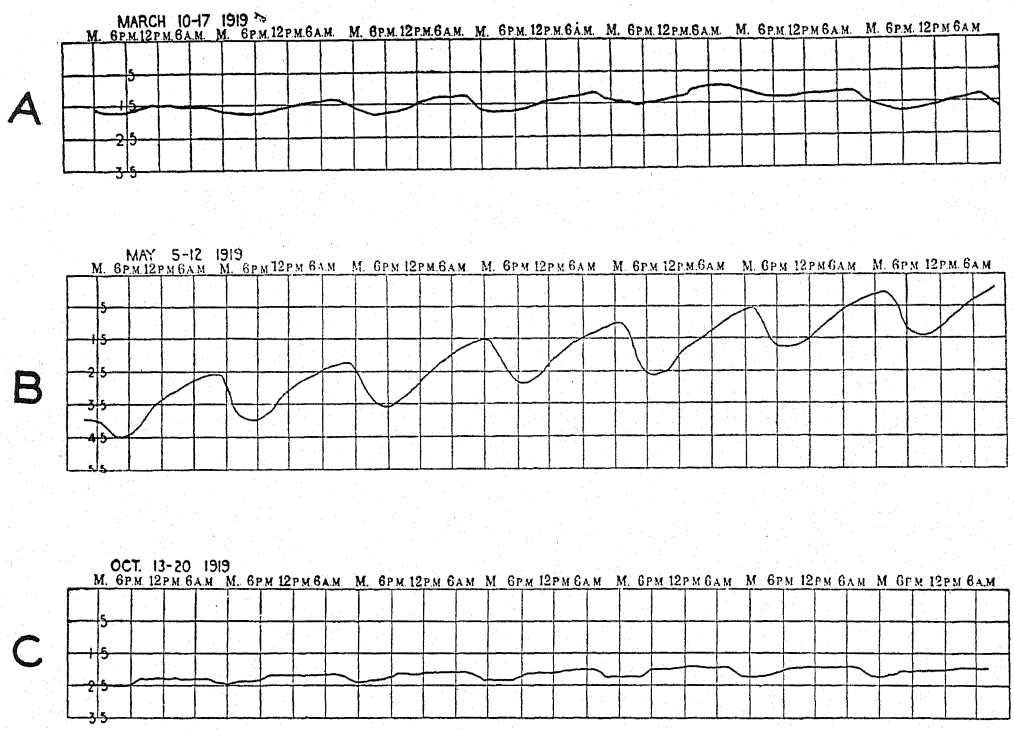


Fig. 1. Dendographic records of variations in thickness of trunk of Arizona ash (*Fraxinus arizonica*) induced by variations in tensions in the hydrostatic system. Reduction in thickness with accelerating transpiration and resulting heightened tension beginning at sunrise. Shrinkage attributable to partial collapse of phloem conduits and loss of water by living cells of phloem, cambium and xylem. Recovery with lessening tension and by osmotic repletion of cell-liquids in afternoon and night.—A. Daily variations March 10–17; inflorescences open but leaf-buds sealed. Slight upward course of tracing denotes initiation of growth.—B. Record for May 5–12. Leaf-surfaces fully expanded. Transpiration, temperature, hydrostatic tensions and growth at maximum. Diametral increase of 4 mm registered.—C. Record for October 13–20. Erratic variations and slight increase in thickness attributable to development of abscission layers in petioles and final stages of enlargement of elements in phloem. Variations ×10. Spaces in grid indicate 10 mm vertically and 6 hours horizontally (adapted from MacDougal, 1921).

While it is true that the terminals of the hydrostatic system are in living cells of roots and leaves, these elements do not vitally participate in the streaming movement of liquids in the conduits, although their osmotic exchanges may play a part in the determination of the composition of the solutes. Thus for example, if a tree is killed without mechanical disturbance of the colloidal material of its cells, reversible variations will be of lesser amplitude than in the living tree, since they consist of depletions and repletions of the water in the colloids of the dead cells (MacDougal, 1921; 1925, p. 71). The wholly physical nature of the flow in such cases is further exemplified by the fact that the direction of flow in conduits is determined solely by existing tensions. Thus Biddulph and Markle (1944) in tracing the flow of radiophosphorus from leaves into stems found both upward and downward flow from the region of entrance into stems. Material passing into the xylem was drawn upward at a rate of 21 cm hourly. The implied downward movement in the phloem, passing radially into the xylem, was characterized as a "circulation" by these authors. (Also see Colwell, 1942, as to use of radioactive reagents.)

The implied translocations and flow of solutions might more accurately be visualized as an eddy or whirlpool (leaf-sieve-conduits-rays-tracheids) tangential to which the main current in the conduits of the xylem would ascend to the leaves, carrying electrolytes and organic compounds coming from the roots, and also leaf-products moving downward in the sieve tubes and drawn through the rays at all levels. Appropriation of portions of the "load" of the main stream would be made by maturing elements of the xylem or by any primary or secondary meristem in organogenic connection. Inflorescences might be regarded as blind laterals or sinks into which metabolites would flow to be used in the morphogeny of flowers and the maturation of fruits and seeds, from which no return to the metabolic cycle of the parent plant could be made.

Metabolites produced by the photosynthetic processes in green leaf-cells move from the tracts in which they originate to the conduits in the phloem, chiefly by converting the energy-rich compounds to compounds with a lower energy level. The phosphorylated carbohydrates and proteins built up in the leaves would flow into the phloem in which a high rate of respiration prevails, in accordance with the

principle that material in tracts under hydrostatic tension would be withdrawn by living cells in which use or conversion occurs.

An analogous procedure had been found by MacDougal and Dufrenoy (1944) in mycorrhizal roots. The migration of material from the soil or through the hyphae is based upon and begins with the formation of ester linkages with inorganic phosphates forming energy-rich phosphate compounds by oxidation-reduction. Catalytic action would result in rearrangement of the bonds and translocation of the products in regions of high oxidase activity, especially in the endodermis and pericycle where catechols are precipitated and unstable energy-rich phosphates are accumulated in the stele to be translocated as above.

The foregoing generalizations pertain chiefly to the flow of solutions exemplified in large plants such as trees, in which nutritive material may enter, be synthesized or hydrolyzed in tissues more than a hundred meters distant from regions of utilization or conversion.

The principal features of translocation, with the more important anatomical details, of *Pinus* and *Sequoia* are described in the following section. New experimental results supporting previous observations confirmatory of the cohesion theory of movements of liquids are given.

Translocation in Pinus radiata.—A clear understanding of the migration of solutes entails a consideration of anatomical details of phloem, rays and tracheids in connection with the seasonal program of development and growth.

The apical meristem begins advance in the spring, developing leaf and branch primordia laterally and secondary meristem or cambium below, with the ensuing program of differentiation of xylem inwardly and phloem outwardly, certain elements of which function as conduits upon maturity. A scheme of growth prevails by which the traces of the new leaves are inserted on the xylem of the previous year, and these organs become important as transpiratory sinks and photosynthetic centers by midsummer. The total outer or epidermal surface of the three suites of leaves of a Monterey pine may amount to as much as 500–600 sq. meters, but the walls of loosely arranged parenchyma in the interior provide vaporizing surfaces of an extent that may be estimated in hectares or acres. These moist walls are the summit of the hydrostatic system.

The conduits in which the most marked flow occurs are in the form of a hollow cylinder 1–5 cm in thickness. The rays which are in effect sheets of pitted parenchyma cells and short tracheids extending radially through the cambium are one to three cells thick and as much as 15 cells high. The rays are separated from each other by about eight long, erect tracheids on the average. Every element of a ray connects through one to three pitted openings with the tracheids with which it is in contact.

The cambium initials and their derivatives which are still capable of growth include 10 to 20 layers of turgid thin-walled elements of a length slightly less than that of the elements into which they finally differentiate. A similar anatomical arrangement prevails in the roots subsequent to the exfoliation of the cortex during the first season of growth.

Occurrence of carbohydrates.—The sparse formation of starch and the ease with which determination of the sugars in the conduits may be made afford opportunities for the appraisal of movements of material in the hydrostatic system in normal trees and under experimental conditions. The supposedly phosphorylated sugars moving downward in the conduits of the phloem are drawn through the rays into the xylem. Some of this material is condensed into starch in the parenchymatous ray cells, but the greater part passes into the xylem in soluble form under normal conditions. Synthesis of starch may be induced in the xylem by girdling (MacDougal, 1943).

In June, 1925, at which time photosynthesis would have been at a maximum, the outermost layer of tracheids, not yet mature and retaining some protoplasm, contained sap with a sugar content of 0.27 g per liter estimated as glucose, with pH 5.6. The second and third layers in which the upward movement of sap would be at a maximum had a sugar content of 0.16 g per liter at pH 5.4. A second sample two days later showed 0.20 g and 0.07 g per liter respectively. From these and other determinations it was seen that the relative concentration of sugars is least in layers in which the maximum upward flow of sap occurs (MacDougal, 1926, p. 79).

The outermost layer on July 2, at which time cambial use of material was nearing zero, had a sugar content of 0.49 g per liter; a few days later the second and third layers showed 0.28–0.30 g per liter. In consequence of a further inactivation of the cambium with lessening use of carbohydrates, the concentration in these two inner layers had risen by August to 1.4 g per liter. In the last week in October, at which time some increases in volume identifiable with growth were observable especially in the phloem, the concentration of glucose in the woody layers had fallen to 0.20 g per liter.

The loss of the oldest suite of leaves by autumnal leaf-fall in late September together with lower temperatures would reduce the supply of leaf-products passing into the phloem. Coincidental lessened transpiration would result in relaxed tensions in the hydrostatic system and a slower rate of movement of sap. Initiation of growth in the January-February period would increase the utilization of building material. Near the end of the season of cell-division, enlargement and maturation, a balance is reached after which time a surplus of carbohydrates accumulates, as exemplified by the estimations given above.

Interpretation of the effects of defoliation.—Defoliation removes the vaporizing surfaces, the loss of water from which sets up tensions in the hydrostatic system, abolishes the main photosynthetic mechanism and also modifies the production of

auxins to an extent not yet determined. These effects are especially marked in the Monterey pine and vary widely with the seasonal stage in which the operation occurs.

This tree in its native habitat carries three suites of leaves during the summer season of active growth; the oldest is cast off by October, after which photosynthesis is carried on at a lower rate until March or April. Only a sparse surplus of carbohydrates is accumulated at any time; some starch may be seen in the younger parts of the rays. Experimental removal of all leaves in the period of least activity in December gradually reduces the streaming movement from the roots to the terminals in the xylem as the tensions are relieved. The supply of leaf-products to the phloem being at an end, the amount in transit, never very large, is soon exhausted. The prospective new set of leaves, which normally carry out their earlier development at the expense of surplus material in the xylem and are not "self-supporting" do not reach normal length and furnish but little material for cambium action until mid-season with a resulting defective layer of wood. Defoliation after the new leaves have appeared is followed by death in mid-summer; operations at later dates variously affect survival and subsequent growth (MacDougal, 1936, 1943). All effects are explainable as results of the cancellation of the "circulation" and movement of material from the leaves downward through the phloem.

The effects of girdling.—The removal of a belt of cambium and all tissues external to it stops the downward movement of material in the phloem at the upper margin of the girdle and may modify upward movement in the layer of conduits exposed. This operation does not materially alter the radial extension of the hydrostatic system, either above or below the girdle, but some notable reactions follow the interruption of the movement of material in the phloem at the upper margin of the girdle and may modify upward movement in the layer of xylem conduits exposed. Growth continues below the girdle until the supply of leaf-products in the phloem conduits is depleted, usually 14 to 16 days. Stoppage does not imply the death of the cambium, as it may be activated later in the season if the exposed wood is bridged from above by callus tongues, furnishing conduits for renewed flow of auxins and building material.

Girdling in the winter resting season generally results in the death of the tree, but the operation during the growing season may allow the tree to continue activity for one or more seasons, a result probably dependent upon the surplus carbohydrates present, the adequacy of the leaf-system, and upon the activity of the mycorrhizal system. If the conduits in the phloem are blocked by compression without traumatic effects, as has been done with belts of metal plates, a diminished wood formation results below the compression. A further illustration of the action of the hydrostatic system is to be found in the effects of girdling one of twin trunks. Material drawn upward in the xylem from the common unaltered base makes possible some growth below the girdle (MacDougal, 1943).

Girdling in the Monterey pine is followed by the cessation of enlargement above the denuded zone, which is generally resumed in about a fortnight. Adequate explanation cannot be offered, but it is evident that the exposure of the outermost layer of conduits of the xylem and their reduced capacity would be an important factor. The experiments of Bonner (1944) with girdled tomato plants are of interest in this connection. Thiamin, pyrodoxine, pantothenic acid, riboflavin, sucrose, total nitrogen and non-protein nitrogen accumulated above the girdle at varying rates. The possibility of similar action in pines has not been explored.

That carbohydrates are accumulated in the Monterey pine is evident from the heavy deposition of starch in the wood parenchyma in which it is otherwise rarely to be seen (MacDougal, 1943). The accumulation may be due to deficient phosphorylization, as it occurs in injured tissues. Nothing so definite can be said of the exaggerated growth of the parenchymatous cells containing the starch. Hypertrophied parenchyma and surplus starch extend several meters above the girdle.

Radial measurements at various levels of a girdled Monterey pine.—Previous observations had followed the effects of girdling to a distance of 75 cm. above a girdle. A final series of measurements was made on Pine No. 72 in 1944–45. Dendrometers were attached to a root 20 mm in diameter at a point 60 cm from the base of the trunk, which was 20 cm in diameter. Dendrometers were put into operation at various levels on the trunk as indicated in table 1, the uppermost being at a level in which the trunk was 8 cm in diameter with bark which had not begun to flake. Increases in diameter at all levels during 1944 were registered for comparison with growth in the following year in which girdling was performed (fig. 2).

The immediate effects of girdling duplicated reactions were previously recorded (MacDougal, 1943). Cambial action continued below the girdle for a few days only, and the application of alpha-naphthaleneacetic acid in carbowax to the lower margin of the girdle did not extend the period of activity, a result supporting the conclusion that growth below the girdle continued until the balance of nutritive material in the phloem was exhausted.

The reaction above the girdle was also in accord with previous results; enlargement was cancelled by the operation but was resumed a few days later with a seasonal continuance beyond the usual date. A heavy callus was formed at the upper margin of the denuded cone and exaggerated diametral increase ensued, attributable for the most part to the hypertrophied wood parenchyma noted above. Increased growth extended to the level of the highest instrument, 4 meters above the girdle, as illustrated by comparison of the measurements of 1944 and 1945.

TABLE I. *Radial increases in a root and trunk before and after girdling 90 cm above base.*

	Distance above base of trunk						
	Root	10 cm	90 cm	130 cm	210 cm	300 cm	420 cm	560 cm
Radial increase at levels indicated: 1944								
	3 mm	6.0	5.1	4.6	5.2	9.3	6 mm	..
Radial increases previous to girdling in mid-May: 1945								
	3 mm	2.6	3.5	3.0	3.1	3.0	4.6	2.5
After girdling:								
	0.0	0.0	0.0[a]	2.3	2.8	3.5	2.4	3.8

[a] Location of girdle.

A total cessation of growth came in August, after which a shrinkage of the trunk ensued; the loss immediately below the girdle was 2.5 mm; immediately above the girdle it was 0.2 mm, while at the uppermost instrument the radial contraction was 0.7 mm by October 31. November was characterized

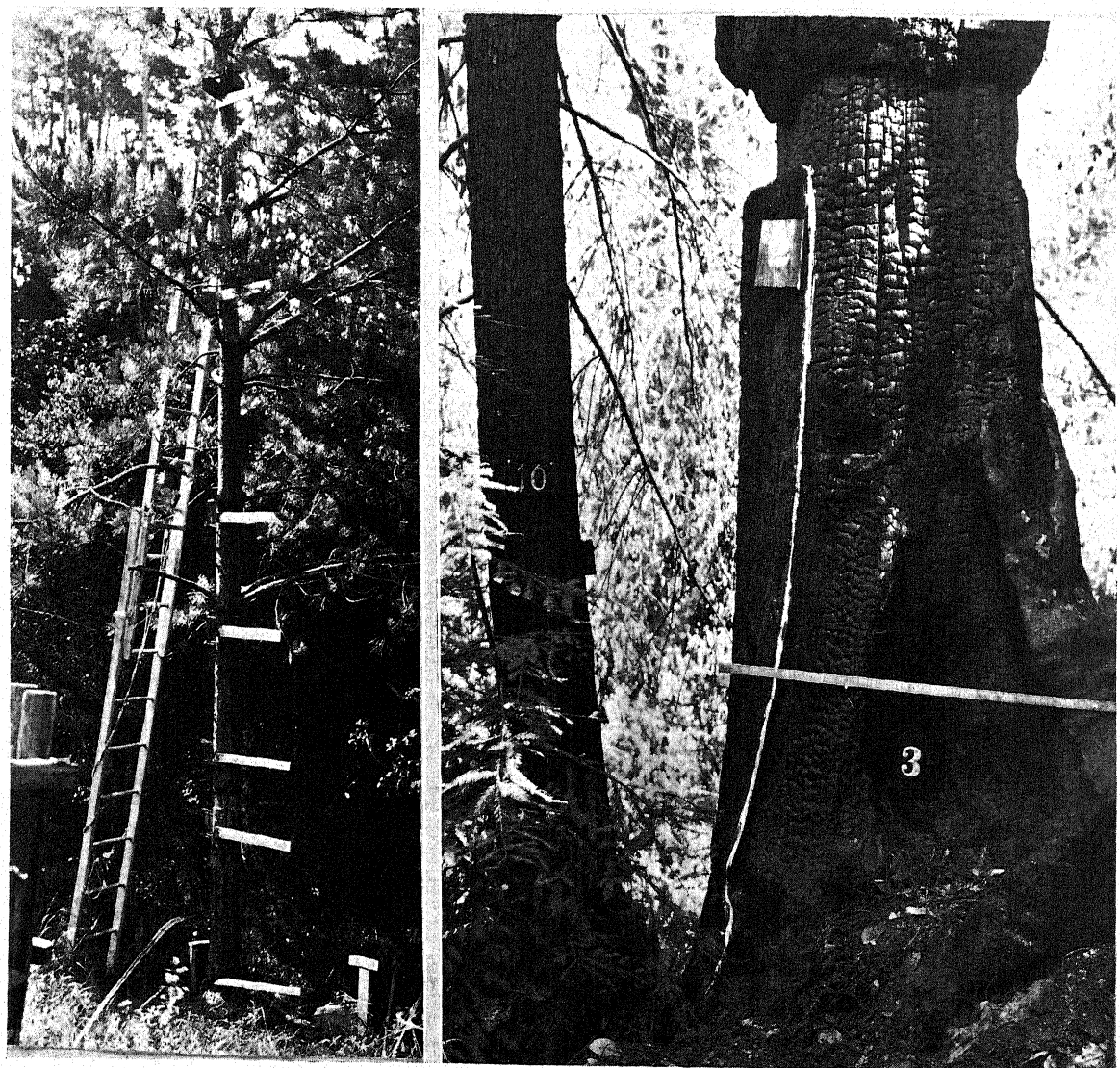


Fig. 2 (Left). *Pinus* No. 72 with dendrometers attached to root and at seven levels as indicated by white markers. —(Right). *Sequoia* No. 27C subsequent to forest fire in 1941.—2. Upper part of sector kept alive by nutritive solutions received from young trunk (10) until burned in 1941. The white curved line is the chalked callus margin of this tract.—3. Central part of the trunk burned by fire. Dimensions indicated by meter bar above "3" (see fig. 2).

by the occurrence of many showers with intervening periods of warm sunny days.

The increased supply of moisture brought no change below the girdle; shrinkage continued above with further loss in radial measurements of 0.3–0.6 mm by the end of November. The leaves of the young suite were of normal length but autumnal leaf-fall was incomplete, suggestive of a deficiency of auxins necessary for the development of abscission layers. An abrupt yellowing ending in death of all leaves followed. These organs remained in place, however, so that the main anatomical features involved in the hydrostatic system were intact. Daily variations in tensions with slight reversible changes in diameter were registered by a dendrograph attached to the trunk below the girdle. These measurements confirmed the endurance of a reduced hydrostatic system in dead trunks as previously reported (MacDougal, 1921, 1925).

Complementary arrangement of the hydrostatic and pneumatic systems.—The part of the hydrostatic system which is in non-living conduits sustains complementary relations to the pneumatic system. The atmospheric gases in intercellular spaces, vessels, tracheids and old parenchymatous elements in a mature trunk of pines have a total volume equivalent to about two-fifths of the overall volume of the trunk. The proportions in which these gases are present are invariably different from those in the atmosphere. Thus carbon dioxide which usually is found in only 2 to 3 parts in 10 thousand in the air may constitute 1,250 parts in the pneumatic system of the Monterey pine, an excess which may reasonably result chiefly from metabolic processes, although a portion might be freed from the ascending sap current. Oxygen is all but invariably in smaller proportion than in the air, although in other trees, such as the oak, samples have been obtained containing an excess of this element. The proportion of nitrogen varies widely. The body of gases of the pneumatic system are in direct connection with the air by the intercellular spaces in the rays extending through the cambium and phloem. Connection is made through the lenticels in some trees, but in pines the passages are in the outer non-living elements of the phloem. Although the channels are not well-defined, it was found that an average rate of flow of 0.004 cc per sq. cm. per hour takes place under a difference of pressure of 100 mm HG (0.32 atm.) (MacDougal and Working, 1933).

The fluids of the two systems may occupy vessels and spaces alternatively. Under conditions of drought with an excessive loss of water the heightened tensions may pull sap out of some conduits with the consequent entrance of gases.

Diminished tensions in the hydrostatic system notably resulting from autumnal leaf-fall or defoliation experiments may be followed by the reoccupation of vessels and spaces by capillary action, which would compress the gases so that attached manometers would register positive pressures. Such pressures would be relieved by outward streaming through the rays, as exemplified by the measurements given above. Injections of liquid into spaces normally occupied by gases would in a similar manner result in registrations generally designated as "root-pressures."

It is also to be noted that the coefficient of expansion of gases might account for the bleeding of cut surfaces of trees in the rising temperatures of spring following winters of extreme cold. Heightened metabolism and specialized anatomical features, such as the vasicentric wood-parenchyma of the sugar maple may play a part in the marked flow of sap in this tree (Stevens and Eggert, 1945). Experimental results from isolated sections of stems or trunks may be as indeterminate as in "root-pressures."

TRANSLOCATION IN THE COAST REDWOOD (SEQUOIA SEMPERVIRENS).—The general scheme of the hydrostatic system and its action in the coast redwood is in conformity with the outline given in the opening section of this paper. Similarity to the pines consists in the retention of several suites of leaves with the loss of the oldest suite by autumnal leaf-fall and the maintenance of upward movement of sap in the conduits directly connected with the leaves (3 to 5 suites).

Divergent features include extensions of the hydrostatic meshwork in the older wood with injection in the intercellular spaces, a heavy surplus of starch, much of which remains unconverted in wood of great age, and the endurance of many parenchymatous cells in the living condition in the wood more than a century old. These trees may survive to an age of 10 to 15 centuries, building massive trunks with a height of over a hundred meters. As a consequence some material from root-terminals may be carried 150 meters from the place of entrance or synthesis to the terminals in the crown. A marked regenerative capacity results in the organization of domes of primary meristem in the endodermis and pericycle of the parent tree.

Newly formed xylem has a glistening white appearance, which is retained for 20 to 25 years; this heavy layer of sapwood then passes into the condition of reddish brown heartwood, in which many parenchymatous cells endure to a great age, as noted above. The watery content of the heartwood is very high and its movement in response to varying tensions in the hydrostatic system is facilitated by abundant pits.

Effects of girdling.—The immediate reaction to girdling is similar to that of the pines, consisting of a temporary cessation of growth above and an uninterrupted enlargement below, which comes to an end, however, in a fortnight. The inactivated cambium may renew growth if the girdle is bridged by callus tongues from the upper margin of the girdle within the season.

Perhaps the most notable effect of girdling is that in which the layers of exposed sapwood begin desiccation immediately and the underlying 20 to

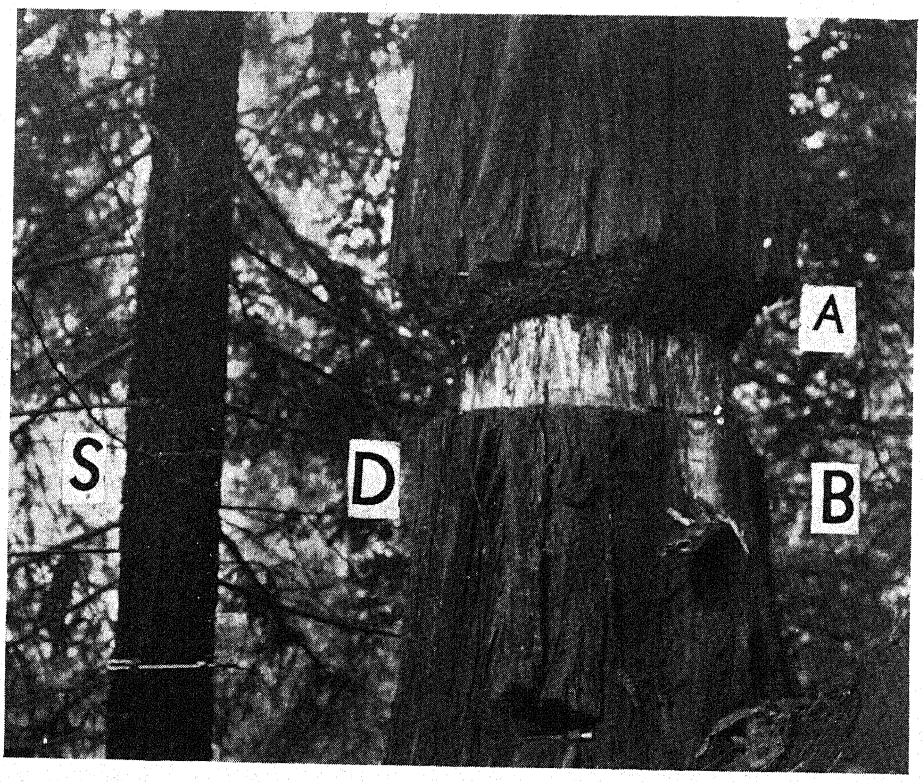
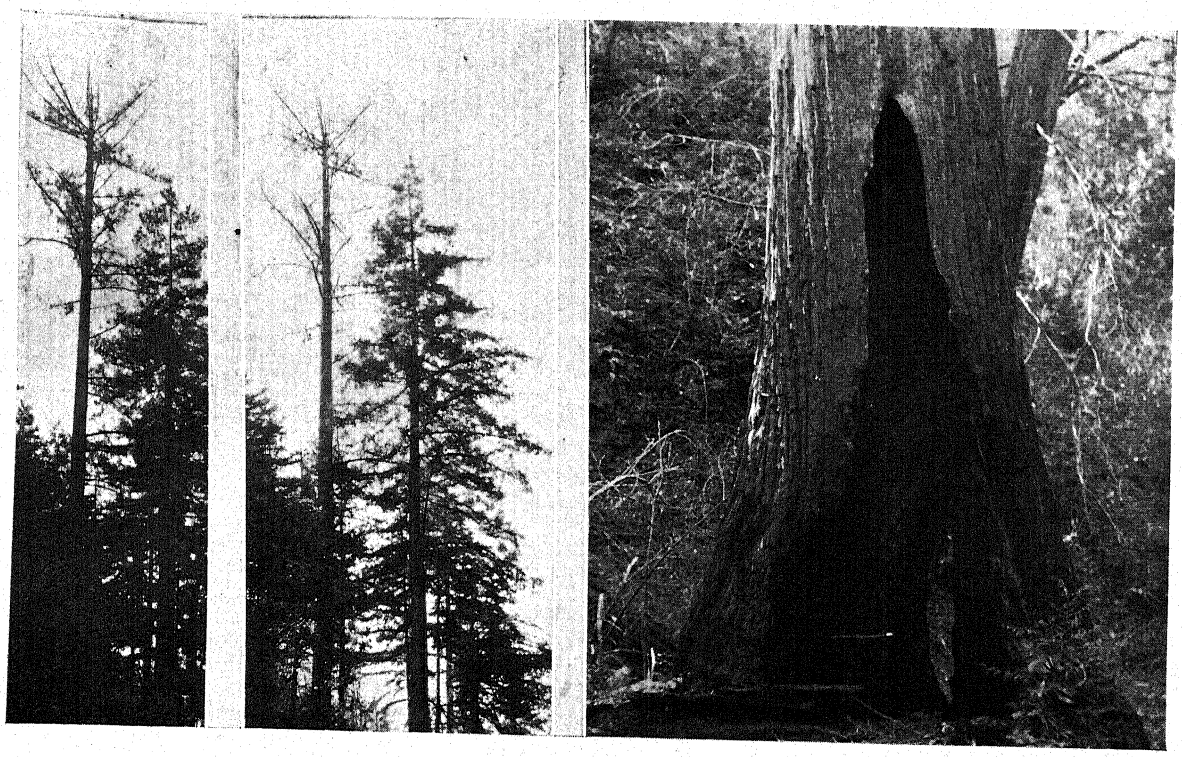


Fig. 3. Trunk of *Sequoia* No. 27C, girdled January, 1920, photographed February, 1939. Small trunk (S) at left developed from cambium of connecting root. Heavy callus (A) above girdle. Exfoliating bark (B) external to dead sapwood. Active cambium and living sapwood in sector D connecting with large root: 25 layers of sapwood in denuded zone of girdle (see fig. 3). Reproduced from MacDougal, 1939, by permission of Amer. Philos. Soc.



25 layers become dry and brittle by the end of the season. All upward movement in the hydrostatic system through the girdled zone must take place in the heartwood.

Results of girdling of many trees have been previously published (MacDougal, 1938). Further details with regard to one previously designated as No. 27C are given below. This tree was girdled in February, 1920, and carried green leaves until August, 1945, having endured 26 seasons subsequently (figs. 3, 4, 5).

A sprout arising from a large root at a point about a meter from the base of the trunk about the time of girdling had made such development that it had the stature of a small tree, 25 meters in height with a diameter of 25 cm at the base at the time of the death of the main trunk in 1945. The trunk had a sheathing layer of dead sapwood below the girdle during the long period of observation, except in the sector vertically connected with the young tree, which comprised about one-third the entire circumference. This condition prevailed from 1920 to 1941, during which time the translocation of material necessarily followed the pattern illustrated by figure 5.

The downward flow of leaf-products in the phloem of the young trunk and in the old trunk above the girdle was not disturbed. Leaf-products and mycorrhizal derivatives would be drawn to the base of the old trunk as indicated at U, and upward in the phloem below the girdle, below which solutions necessarily passed inward through 25 layers in the early stage and 50 layers of wood at the close to move upward in the xylem.

Movements of solutes upward in the xylem of the young tree would follow the usual course. Some of its leaf-products, however, on reaching the base of the trunk and passing into the large root would become subject to the hydrostatic tensions of the larger tree and flow upward in both the xylem and phloem in the sector connected with this root. The amount carried in the phloem was adequate for the activities of the cambium. Theoretically some of this material would be carried past the girdle and become available for respiration or growth if it reached living cells.

Experimental conditions were intensified by a forest fire in October, 1941, by the action of which all of the dead sapwood below the girdle and the older wood was burned away as illustrated in figure 3. The sector with living phloem was killed but the trunk of the young tree, which was scorched, was not injured beyond the loss of a few lower branches.

The only conduits now available for the upward flow of material were those in the older wood which had been reduced to half of the original cross section. The solutions passing upward were sufficient in volume to maintain a diminishing number of green leaves until August, 1945, at which time the remnant was cast off. The conditions for conduction in this were directly opposite to those described in the next section.

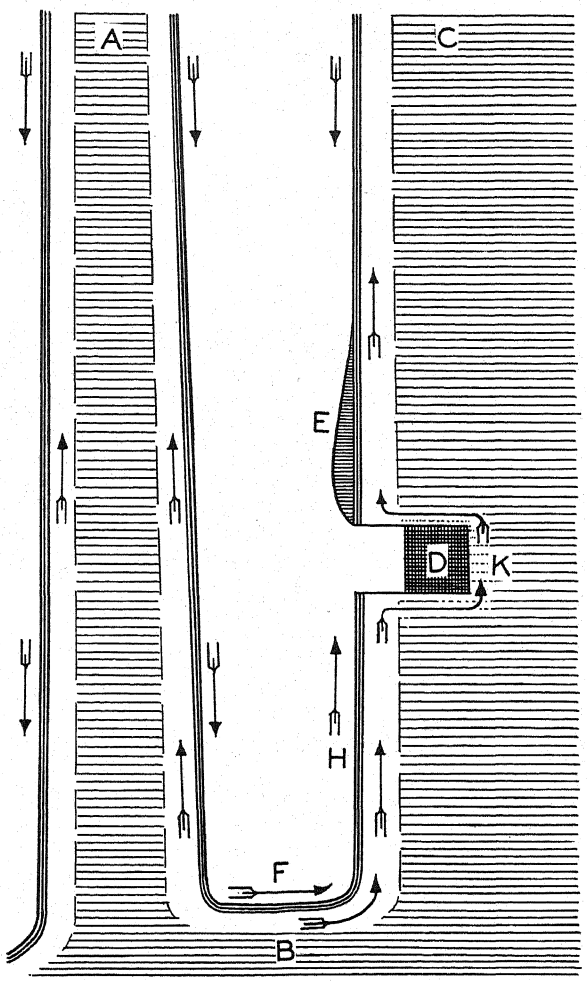


Fig. 5. Diagram of flow of solutions in *Sequoia* No. 27C and in accessory trunk.—A. Longitudinal section of small trunk.—B. Upper part of large root connecting the two trunks.—C. Longitudinal section of outer region of main trunk facing small trunk with which it is directly connected by root B. Phloem is represented by heavy parallel lines, sapwood by clear spaces, and old wood by horizontal lines.—D. Dead sapwood in girdled zone.—E. Callus at upper margin of girdle. The upward courses of sap flow in sapwood and older xylem is denoted by arrows. Downward flow of solutions in phloem similarly keyed. Special translocation from the base of the small trunk through the root at F and upward in the phloem below the girdle at H is indicated. Ascent of sap past the girdle is through woody layers 25 to 50 years old.

Translocation in hollowed trunks.—Trees of many species with the central parts of the basal region removed by fire or decay are of common

Fig. 4 (Left). Summit of girdled redwood, 27C, July, 1939, with normal tree at right.—(Center). Girdled redwood August, 1945, in the final (26th) season; the small number of leaves present were lost in the following month.—(Right). Basal region of *Sequoia,* the central part of which was destroyed by fire 48 years earlier. The three basal segments are separated by wide intervals, one of which appears in the photograph (photo, August, 1945).

occurrence. Many small trees of several species may be found in which as much as four-fifths of the basal part of the trunk is of dead and desiccated wood, the living part of the trunk being in the form of a thin shell of recently formed xylem and phloem in a narrow sector, through which all translocated material from leaves and root systems must pass.

Schemes of translocation prevalent in such structures offer modifications of the hydrostatic system, by which the conduits for the free flow of upwardly moving material are greatly reduced, with the reserve in older wood absent. Extensions in the phloem remain unchanged, but the phloem may be reduced in accordance with its circumferential measurements.

A redwood tree so altered stood near 27C and an analysis of the possibilities of translocation was made. The basal flare of the trunk was represented by three thin buttresses comprising about two-thirds of the circumference, in which phloem was maintained normally. The wood internal to these thin buttresses which had been destroyed by fire and decay would have made a cone with its apex 7 meters up in the center of the trunk, 3 meters in diameter at the base with a total volume of about 16 cu.M equivalent to one-third the total volume of the whole trunk (fig. 4).

In addition to the normal number of layers of sapwood, the part of the heartwood remaining was about 15–20 cm in thickness, in which the moisture content was very low, so that it could be assumed that the hydrostatic system did not effectually extend beyond the sapwood.

Anatomical evidence was to the effect that destruction of the central cone had occurred in 1898; the chief feature being the fact that the annually formed layers of wood previous to that date were 2–3 mm in thickness, while those of the 47 seasons including 1945 were less than 1 mm in thickness.

The total foliar expansion appeared to be normal so that the tensions set up in the hydrostatic conduits would be those of a normal tree. The possible upward flow in the xylem would have been restricted, however, with the disappearance of the great surplus in the old wood. The supply of material from the roots would be reduced, with an inevitable diminution in the number and size of the xylem elements formed in contrast with girdling effects in which conduction in the phloem and the outer xylem is interrupted. Such effects are cumulative and bring about the death of trees such as a pine within a season or two.

Discussion.—The play of forces which brings nutritive material into regions of use and conversion includes identical factors with widely disproportionate importance in various types of shoots. Translocation of material in small herbaceous annuals with a life cycle of a hundred days necessarily follows a pattern different from that of a tree with a towering trunk in which material must move more than a hundred meters from absorption surfaces to regions of conversion or use.

Movement in the hydrostatic system by which enormous quantities of solutions are lifted to the vaporizing surfaces of the leaves is powered by energy derived directly from solar action. The proportion of the energy thus acquired expended in vaporization is estimated as high as 98 per cent by some authors (Compare Transeau, 1926).

A small fraction of radiant energy trapped in the leaf-screens activates the photosynthetic processes. Streaming translocation in the non-living conduits carries the metabolites synthesized in the leaves to regions of conversion and use. Compounds absorbed through the roots are similarly transported to within short radii—not greater than a millimeter or two—of the living cells in which they are used. Withdrawal from the hydrostatic system is by energy stored at high levels in these compounds, liberated by respiratory processes at the boundary phases of vacuoles in the cells into which the metabolites pass by osmotic exchange or diffusion.

Movement in the non-living conduits and in the living cells all but invariably transports nutritive material from high to low energy levels, from places of synthesis or storage to regions of conversion or use in meristematic tracts, inflorescences, fruits or surplus reservoirs. Stated otherwise, the flow is from source to sink and this conception may be extended to the ascent of sap in which soil solutions move to transpiratory sinks in the foliar organs, from a region of high vapor pressure to one of low vapor pressure, which also applies to all nutritive material. A realization of these fundamental features renders superfluous theories of mass flow or polar transport of nutrients of accessory substances such as auxins, vitamins or hormones.

Morphological features are without doubt of great importance in the pattern of hydrostatic, osmotic and diffusion movements in any tree. Thus the upwardly moving sap stream in *Salix* has been found to occupy the summer wood of all layers of wood in mature trees; the spring wood of all annual layers of *Alnus* furnishes sap conduits while in *Juglans* sap ascends in the late summer wood and early spring wood (MacDougal, Overton and Smith, 1929), while the most recently formed annual layer includes the path of the ascent of sap in the chestnut. Correlative schemes of leaf-insertion and of radial connections are involved.

The stage at which streaming movement in conduits may be induced by hydrostatic tensions has been determined in only a few species. In the phloem the sieve tubes become channels of rapid translocation early in the season and the period over which flow may take place in any individual element is brief and may end before cessation of seasonal growth (Crafts, 1939).

Streaming movements do not take place in the layer of tracheids of the Monterey pine formed in any season until the connecting protoplasm strands have disappeared from the perforations in the membranes of the pits. Whatever flow may take place

during the remainder of the season is that which may be induced by tensions communicated laterally from the wood of the previous season. Not until the initiation of growth in the following season, when traces of newly developing leaves are inserted on the terminals, are tensions exerted directly on sap in this layer.

This radial flow of liquids, especially in the medullary rays, appears to be characteristic of the pines and may prevail in other conifers, but did not take place in the three dicotyledonous trees, *Salix, Quercus* and *Juglans* examined by MacDougal, Overton and Smith (1929). Radial movement of sap in such trees could take place only by diffusion and in accordance with osmotic differentials. The principal connections of sap in older layers of wood are at the summit, where the meshwork of filaments fuse and the tensions set up in the leaves inserted on the younger layers may thus be communicated to the sap in all layers of wood.

The results of all available observations show that tangential movement of liquids takes place at a very low rate. Substances entering the base of the trunk move upward in the wood in direct connection. Reagents introduced have been seen to occupy an upwardly gradually widening sector of the trunk indicative of a rate of tangential movement or diffusion much less than that of the axial flow. This disproportion may be attributed partly to the fact that the tensions are practically equivalent circumferentially. It is also to be noted that the number of perforated membranes to be crossed in a given distance tangentially is many times that which would be encountered in radial and axial flow. The importance of this last named condition has been confirmed by the limited movement of reagents transversely to the xylem under experimental pressures.

SUMMARY

The filaments of liquid in the hydrostatic system of plants may be visualized as a cohesive meshwork occupying conduits in the xylem, rays and phloem and extending through the moist walls and membranes to the vacuoles of all or nearly all living cells of the plant, including the apical meristem and the cambium with its immature derivatives.

Tensions set up in the system by vaporization from cell-walls in the leaves cause a flow of many cm per hour in non-living conduits, such as tracheids, vessels and sieve tubes and withdrawal of water from living cells.

Increasing tensions result in reduced diameter of non-living elements with collapsible walls and of volume of turgid cells. These variations are greatest in the phloem of the trees examined. Abrupt contractions resulting from accelerated transpiration at sunrise continue for 5 to 7 hours and are relieved the following 17 to 18 hours beginning after midday by osmotic action with lessened tensions.

The course of movement in the conduits is in the form of a whirlpool, in which the ascending sap constitutes the main stream with the downwardly flowing leaf-products being drawn in radially or laterally at all levels to be carried upward. Inequalities of conversion may be followed by upward movements in the phloem.

The supposedly phosphorylated carbohydrates formed in the leaves are translocated as such in *Pinus radiata*. Condensation into starch occurs only in the rays. The concentration estimated as glucose is highest in the outermost layer of wood, and least in the conduits in which the most rapid movement of sap takes place. Seasonal variations consonant with photosynthetic activity and growth are recognizable.

Girdling of *Pinus* cuts a sector of the phloem out of the circulatory system, and the leaf-products in the region below the girdle are depleted by being drawn into the xylem. Inactivation of the cambium follows. Growth may be resumed if the supply of leaf-products is renewed by translocation across the girdle through callus bridges.

Traumatic reactions to girdling include the formation of callus at the upper margin of the denuded zone, inhibition of growth for several days, and a subsequent heavy accumulation of starch in the hypertrophied wood parenchyma in a segment of the trunk known to extend 4M above the denuded zone in the Monterey pine. No analysis has been made of the auxins and accessory substances which would necessarily accumulate above the girdle as has been found in other plants.

The effects of defoliation of pines, which vary with the season in which the operation is performed, are attributable to stoppage of photosynthesis and reductions in movement in the hydrostatic system by relaxed tensions.

The immediate effects of girdling of *Sequoia* are parallel to those of *Pinus* in the earlier stages, but later 20 to 25 layers of exposed sap-wood of *Sequoia* desiccate rapidly and are in a dry and crumbling condition by the end of the season. Subsequently the upward movement of sap, which normally takes place in the outer 4 or 5 layers directly connected with the leaves, is in heartwood through the girdled zone.

The scheme of translocation is delineated by which leaf-products of an accessory trunk are conducted basipetally in a large root and upward in the xylem and phloem to the lower margin of a girdle (fig. 5).

Growth in trunk and crown took place at a gradually diminishing rate during 21 seasons after girdling of a *Sequoia*. Half of the wood below the girdle was burned away at the end of this period, but some green leaves were maintained for a further period of four years, which may be taken as a maximum of endurance.

A large *Sequoia* in which fire had destroyed a cone of heartwood in the basal region, equivalent to about one-third of the total volume of the trunk, gave opportunity for observation of effects of a

fortuitous operation complementary to girdling. The outer wood and phloem were in the form of segments of a thin shell. Continuing growth at a reduced rate after 48 years was noted.

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BRAZILIAN CHYTRIDS. IX. SPECIES OF RHIZOPHYDIUM [1]

John S. Karling

RHIZOPHYDIUM IS the largest known chytrid genus and includes at present approximately sixty valid species which are world wide in distribution and ubiquitous in host range. They have been found in tropical as well as in subarctic and temperate regions and may occur as parasites or saprophytes on protozoa, Proteomyxa, infusoria, rotifers, helminths, nematodes, chytrids, Oomycetes, Ascomycetes, freshwater and marine algae, spores of liverworts, mosses and ferns, and pollen grains of gymnosperms and angiosperms. Of the numerous chytrid genera collected by the author (1944a–d, 1945a–c) in the Amazon Valley in Brazil, none was better represented in number of species than *Rhizophydium*.

So far only four species of this genus have been reported from Brazil. In 1943 Viegas and Teixiera described an unidentified species on *Spirogyra,* and the author (1944d, 1946) reported the occurrence of *R. globosum* on the alga *Xanthidium subhastiferum,* and *R. gibbosum* and *R. zoophthorum* as parasites of rotifer eggs. Since that time several additional species have been found, many of which are new or particularly noteworthy. Three of these are hyperparasites of other chytrids, Zygomycetes and Oomycetes; three parasitize microscopic animals, four attack algae, and three occur as weak parasites or saprophytes of pollen grains and dead tissues of higher plants. The developmental cycle of all these species is so similar to that of other known members of *Rhizophydium* that detailed description of this process is unnecessary. The present contribution will thus be limited to a discussion of the distinguishing characteristics, distribution, host range, and diagnoses of the new species.

The most conspicuous of the three hyperparasites noted above developed on the sporangia and resting spores of *Karlingia rosea* and *K. granulata* (fig. 1–4) which were growing on bits of cellophane and onion skin in watered soil cultures from Saõ Carlos, Matto Grosso. Later two similar parasites developed on the sporangia of *Septochytrium macrosporum* (fig. 9, 10) and *S. plurilobulum* (fig. 11–13) in the same watered soil cultures, and although these parasites differ in some respects from the one on *Karlingia,* it is probable that they are all identical or closely related. All attempts to transfer these parasites to other chytrids, water molds, and algae have failed, which suggests that they may be very limited in host range. Thus, as far as hosts are concerned, they differ from other known species of *Rhizophydium*. For this as well as for morphological reasons, these parasites are regarded as representatives of a

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new species, for which the name *Rhizophydium hyperparasiticum* is proposed.

RHIZOPHYDIUM **hyperparasiticum** n. sp.—Fungus parasiticus; sporangiis sessilibus, hyalinis, laevibus, sphaericis (7–42 μ), vel ovalibus (7–15 × 10–24 μ); zoosporis sphaericis (2.5–3.5 μ); sporis perdurantibus hyalinis, levibus, sphaericis (5–10 μ), vul ovalibus; modo germinationis ignoto.

Thalli, numerous, up to 80 on a host sporangium. Sporangia sessile, hyaline, smooth, spherical (7–42 μ), oval or slightly flattened (7–15 × 10–24 μ), with one to several low exit papillae. Zoospores spherical (2.5–3.5 μ), with a minute refractive globule; emerging from one or more of the exit papillae. Rhizoidal system richly branched; coarseness, degree of branching, and length dependent to some degree on size of host sporangia. Resting spores smooth, hyaline, spherical (5–10 μ), oval or slightly angular, with one or more large refractive globules; germination unknown.

Parasitic on the sporangia and resting spores of *Karlingia rosea* and *K. granulata* and the sporangia of *Septochytrium macrosporum* and *S. plurilobulum*, Saõ Carlos, Matto Grosso, Brazil.

The zoospores may attack young as well as mature sporangia and resting spores of its host, and in a few instances as many as 80 small parasites were found on a large sporangium of *K. granulata*. In such cases the parasites are so closely crowded together that they become angular in shape from mutual contact and pressure. As a result of infection, the host protoplasm begins to degenerate and becomes clumped around the rhizoids of the parasites. As degeneration progresses, the refractive material becomes more conspicuous as discrete globules (fig. 1, 9), and by the time the parasites have discharged their zoospores, very little remains in the host sporangium except a few globules and extraneous residue.

As was noted earlier, the parasites found on *Septochytrium* differ in some respects from those on *Karlingia*, and it is not absolutely certain that they belong to the same species. The sporangia on the latter host varied from 7 to 19 μ in diameter and possessed one or two exit papillae, while those on *S. macrosporum* were from 25 to 42 μ in diameter with three to six exit papillae. Furthermore, the zoospores of the latter parasite (fig. 10) were slightly smaller (2.5–3 μ) than those formed by the parasite on *Karlingia* (fig. 2, 3). The difference, however, is so small that it may not prove to be of much diagnostic significance. The majority of sporangia found on *S. plurilobulum* (fig. 11) were slightly urceolate instead of spherical or oval in shape and had two or three exit papillae. The zoospores (fig. 13), on the other hand, were similar in size to those of the parasite on *Karlingia*. Resting spores of the chytrids on *Septochytrium* have not been observed, and it is possible that they may be found to differ from those shown in figure 1A on *Karlingia*. There is thus a possibility that the parasites on *Septochytrium* belong to other species, but until this has been proven, they are grouped under the binomial *R. hyperparasiticum*.

The second hyperparasite to be described here occurred on the conidiophores and hyphae of an unidentified species of *Choanephora* which developed on the surface of moist soil and leaf mold cultures collected in the Acre Territory, Brazil. This species is particularly noteworthy because it is the only known eucarpic chytrid parasite of a zygomycetous fungus. In fact, only two other chytrids, *Pleotrachelus fulgens* and *P. zopfianus* of the family Olpidiaceae, are known to parasitize this group of the Phycomycetes. The species on *Choanephora* (fig. 17, 18) appears to be limited in host range and, as far as is now known, will not attack other chytrids, Oomycetes, algae and pollen grains. Whether or not it will parasitize other zygomycetous fungi is not certain, for at the time it was discovered no other Zygomycetes were available for cross inoculation experiments. In view of the fact that this is the first known eucarpic chytrid parasite of the Zygomycetes and appears to be limited in host range to this group of fungi, the author interprets it as a new species and proposes the name *R. mycetophagum* for it.

RHIZOPHYDIUM **mycetophagum** n. sp.—Fungus parasiticus; sporangiis sessilibus, hyalinis, levibus, sphaericis (7–20 μ), pyriformibus (8–12 × 11–19 μ), 1–3 papillatis; zoosporis sphaericis (3.5–4 μ); sporis perdurantibus sphaericis (6–14 μ), fuscis, rugosis; modo germinationis ignoto.

Thalli numerous, up to 25 on a host cell. Sporangia sessile, hyaline, smooth, spherical (7–20 μ), broadly pyriform (8–12 × 11–19 μ), with one to three exit papillae. Zoospores spherical (3.5–5 μ), with a conspicuous (1–1.5 μ) refractive globule. Rhizoidal system relatively delicate but richly branched. Resting spores spherical (6–14 μ) with a thick, dark brown rough wall, and one to several large refractive globules; germination unknown.

Parasitic on the conidiophores and hyphae of *Choanephora* sp., Acre Territory, Brazil.

The third hyperparasite found in Brazil is *R. carpophilum* (fig. 14–16) which attacks the oogonia of various oomycetous water molds. It developed in two cultures of *Saprolegnia ferax* which had been isolated from a water sample of the Rio Negro at Manaus, Amazonas, and from soil at Campo Grande in southern Matto Grosso. It was later grown successfully on the oogonia of *Achlya flagellata, Dictyuchus* sp., *Thraustotheca clavata,* and pollen grains of *Hevea braziliensis* and *Ulmus americana*. The size and shape of the zoospores, sporangia and resting spores agree closely with those described by Zopf (1884) for *R. carpophilum* in Germany, and there is no evidence at hand to indicate that the Brazilian material belongs to a different species.

Among the three parasites found on microscopic animals, one attacked *Amoeba terricola* in watered soil samples from Saõ Carlos, Matto Grosso. This species is characterized by spherical brown-walled sporangia (fig. 5), oval zoospores (fig. 6) with a

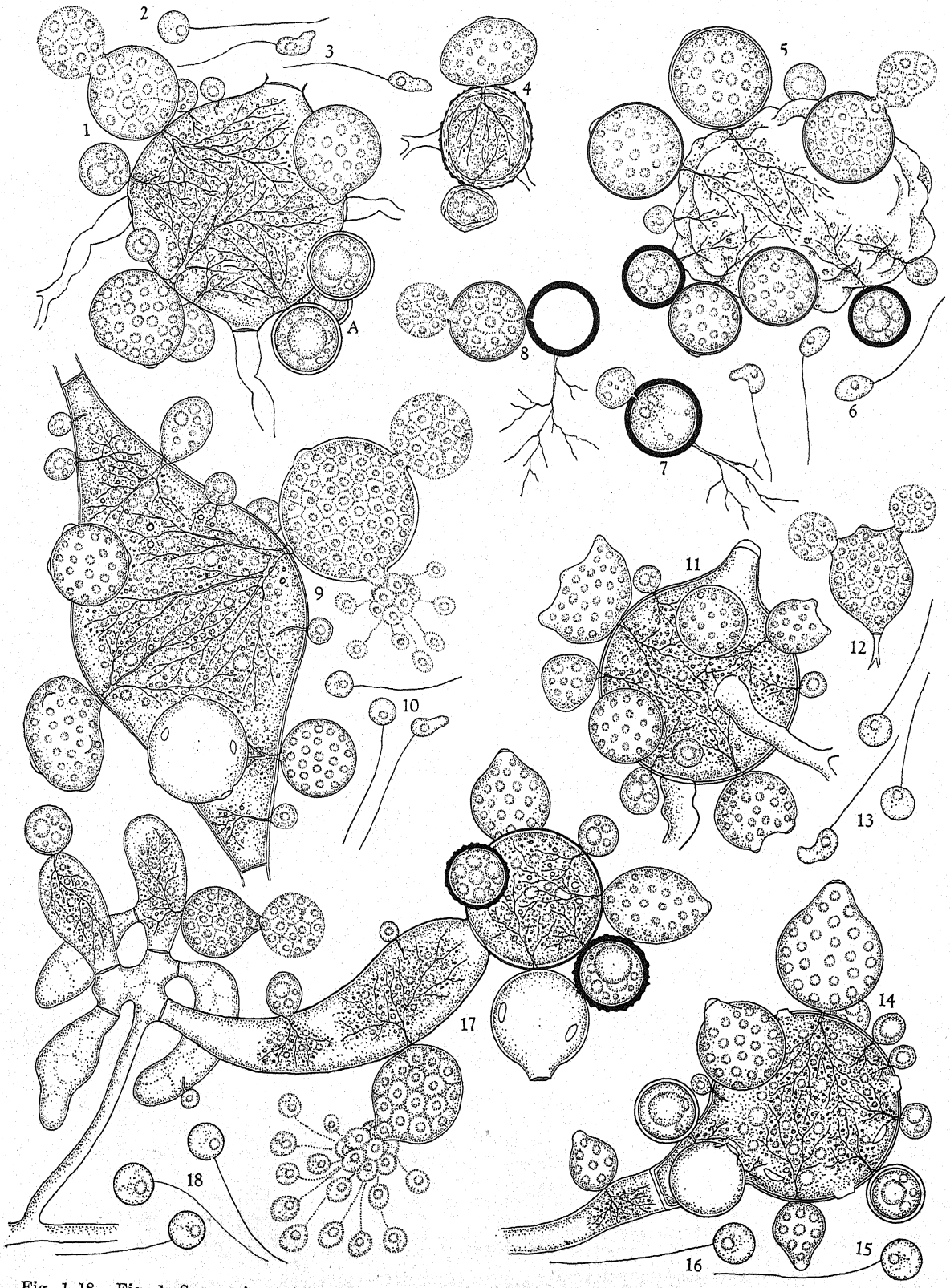


Fig. 1–18.—Fig. 1. Sporangium of *Karlingia granulata* parasitized by 11 thalli of *R. hyperparasiticum;* resting spores at 1A. ×1400.—Fig. 2, 3. Spherical and amoeboid zoospores of parasite. ×1500.—Fig. 4. Parasitized resting spores of *K. granulata.* ×1400.—Fig. 5. *Amoeba terricola* parasitized by ten thalli of *R. Amoebae.* ×1400.—Fig. 6. Oval and amoeboid zoospores of parasite. ×1600.—Fig. 7, 8 Germination of resting spores of *R. Amoebae.* ×1600.—

minute laterally located refringent globule, thick-walled, dark brown resting spores (fig. 5, 7, 8), and is apparently limited in host range to animal substrata. The latter factor, coupled with the color of the sporangia and the oval shape of the zoospores, distinguishes it from other members of *Rhizophydium*. It is accordingly diagnosed as a new species and named *R. Amoebae*. In addition to parasitizing *Amoeba terricola*, it will also grow as a saprophyte on the exuviae of insects, but all attempts to transfer it to fungi, algae, and pollen grains have failed. Its degree of virulence to *Amoeba terricola* is not known, because most of the infected amoebae were dead when discovered. In a few cases, however, the parasitized individuals were still feebly motile. *Rhizophydium Amoebae* may possibly be only weakly parasitic and incapable of attacking healthy individuals of its hosts.

RHIZOPHYDIUM **Amoebae** n. sp.—Fungus parasiticus; sporangiis sessilibus, levibus, fuscis, sphaericis (8–20 μ); zoosporis ovalibus (2 $\times$ 3 μ); sporis perdurantibus sphaericis (8–14 μ), fuscis.

Thalli numerous, up to 14 on a host cell. Sporangia sessile, smooth, spherical (8–20 μ) with a low apical exit papilla and a fairly thick, brown, smooth wall. Zoospores oval (2 $\times$ 3 μ) with a minute (0.5–1 μ), somewhat laterally located refractive globule. Rhizoidal system relatively sparse but frequently branched. Resting spores spherical (8–14 μ) with a thick, dark brown wall and one to several large refractive globules; functioning as prosporangia in germination.

Parasitic on *Amoeba terricola* and saprophytic on insect exuviae, Saõ Carlos, Matto Grosso, Brazil.

The second of the animal parasites occurred on a protozoan in a moist soil sample from Saõ Carlos, Matto Grosso. Only a few specimens of the host and parasite were observed in Brazil, but after the author returned to the United States, he found the same host and parasite in a roadside ditch near Pawling, New York. In the latter collection, the host occurred in great abundance in the exuviae of may flies and was in turn heavily parasitized by this species of *Rhizophydium*. In the United States the parasite appears to attain epidemic proportions in May and disappears in a few weeks, because collections made in the subsequent months contained the host but no parasites. These observations agree with those of Miss Hanson, who noted in 1943 that the parasite disappeared within approximately three weeks in May at New Canaan, Connecticut. Whether or not its occurrence is periodic in Brazil also is not known, because collections were not made at various times of the year. All attempts to grow this parasite on rotifer and may fly eggs, insect exuviae, chitin, bits of corn leaves, onion skin, cellophane, *Equisetum* spores, and pollen grains have failed, which suggests that it may be an obligate parasite. It does not attack the hosts of other known species of *Rhizophydium*. Up to 22 sporangia and resting spores have been found on one host, and the parasite is capable of attacking all developmental stages of the host (fig. 33–35). It destroys and absorbs most of the host protoplasm except a small amount of extraneous residue.

The parasite is characterized by predominantly pyriform sporangia with one to three prominent exit papillae which may often become extended to short tubes (fig. 34). The sporangia may also become spherical, slightly flattened, or somewhat anatropous in shape. The young sporangia almost invariably possess a large refractive globule which gives them a characteristic appearance (fig. 34) until they are of considerable size. Resting spores occur in abundance and are usually prominently apiculate like the sporangia. Because of its limited host range and the characteristic appearance of the sporangia and resting spores, this parasite is interpreted as a new species and named *R. apiculatum*.

RHIZOPHYDIUM **apiculatum** n. sp.—Fungus parasiticus; sporangiis sessilibus, hyalinis, levibus, pyriformibus (10 $\times$ 14 μ) aut leviter obclavatis 1–3 papillis prominentibus praeditis; zoosporis sphaericis (3–3.5 μ); sporis perdurantibus hyalinis, levibus, saepe apiculatis, aut sphaericis (8–12 μ), aut pyriformibus (10 $\times$ 14 μ), aut leviter angularibus.

Thalli numerous, up to 26 on a host. Sporangia hyaline, smooth, fairly thick-walled, pyriform (6–10 $\times$ 14–18 μ), slightly obclavate, flattened, or anatropous with one to three prominent exit papillae which may often become extended to short tubes, 4 $\times$ 6 μ long. Zoospores spherical, 3–3.5 μ, with a small refractive globule. Rhizoidal system richly branched. Resting spores hyaline, smooth, spherical (8–12 μ), pyriform (10 $\times$ 14 μ), often apiculate; containing a large central refractive globule surrounded by several smaller ones; functioning as prosporangia in germination.

Parasitic on a protozoan, Saõ Carlos, Matto Grosso, Brazil; Pawling, New York, and New Canaan, Connecticut.

The third *Rhizophydium* species on microscopic animals occurred as a parasite of the soma and cysts of a stalked protozoan (fig. 19–22) in a soil sample from Flores Nabuco near Manaus, Amazonas. This soil sample had been dried in the laboratory at Columbia University for three months and then watered with sterile animal charcoal water in the usual manner employed in baiting for chytrids. The host and parasite apparently survived the drought successfully because three weeks after the sample was rewatered both appeared in abundance.

Fig. 9. Sporangium of *Septochytrium macrosporum* parasitized by 12 thalli of *R. hyperparasiticum*. $\times$1500.—Fig. 10. Zoospores of parasite. $\times$1500.—Fig. 11. Sporangium of *S. plurilobulum* parasitized by ten thalli of *R. hyperparasiticum*. $\times$1400.—Fig. 12. Emergence of zoospores of parasite. $\times$1400.—Fig. 13. Zoospores of parasite. $\times$1600.—Fig. 14. Oogonium of *Achlya flagellata* parasitized by 12 thalli of *R. carpophilum*. $\times$1400.—Fig. 15, 16. Zoospores of parasite. $\times$1600.—Fig. 17. Abnormal submerged conidiophore and hyphae of *Choanephora* sp. parasitized by 12 thalli of *R. mycetophagum*. $\times$1400.—Fig. 18. Zoospores of parasite. $\times$1600.

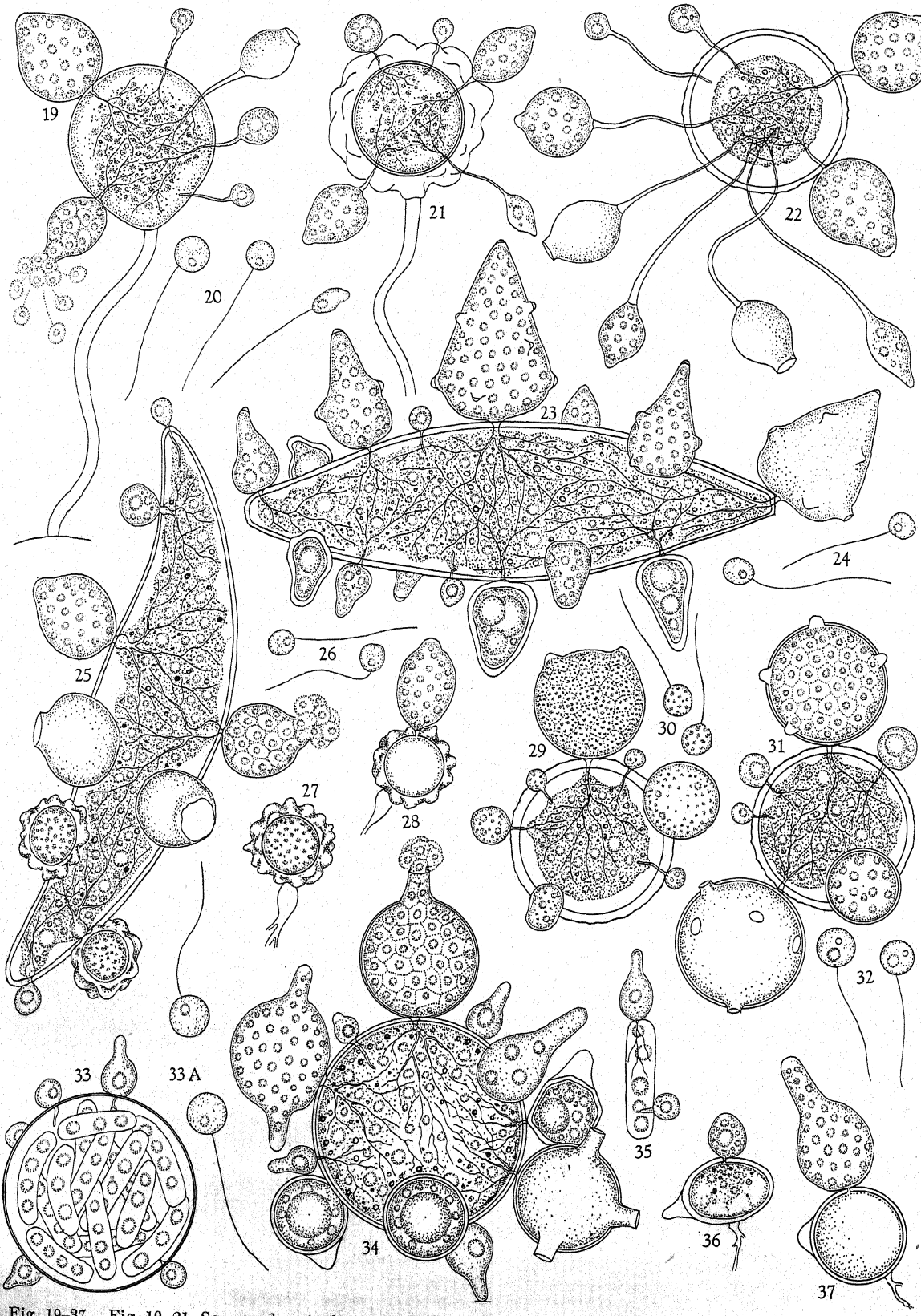


Fig. 19–37.—Fig. 19, 21. Soma and cyst of a stalked protozoan parasitized by stalked and sessile thalli of *Rhizophydium* sp. ×1400.—Fig. 20. Zoospores of same parasite. ×1600.—Fig. 22. Stalked and sessile thalli of same parasite on

In a few cases, up to 20 parasites occurred on a single host, and in such instances the host protoplasm was almost completely consumed except for a small amount of residual matter. This species also grows well on pollen grains of *Ulmus americana* and of other angiosperms. Figure 22 shows a pollen grain of the American elm with eight stalked thalli in various stages of development and maturity. The parasite is characterized primarily by small broadly or narrowly pyriform sporangia which usually stand off from the host on long tenuous stalks. The latter vary from 10 to 50 μ in length and branch within the host to form a sparse rhizoidal system (fig. 19, 21, 22). A few of the sporangia may be sessile and rest directly on the host. When this species was first observed, it was believed that the long sporangia stalk resulted from fortuitous germination of the zoospores at varying distances from the host and the subsequent growth of the germ tubes toward the host cell. Careful study of the developmental stages, however, showed that the germ tube continues to elongate after becoming anchored on the host, whereby the spore body or incipient sporangium is usually pushed farther away from the host or substratum. While stalked sporangia may occur in other normally sessile species of *Rhizophydium,* they are not so constant as in the present species. However, it remains to be seen whether or not the presence of a stalk is a distinct species characteristic. In view of this and the fact that resting spores have not been found, the author does not feel warranted to describe it as a new species.

Rhizophydium globosum was the most common algal parasite encountered in Brazil. It was first found on *Xanthidium subhastiferum* in the swimming pool of the Bosque Club at Manaus and later collected on *Netrium* sp., *Closterium* sp., and *Spirogyra* sp. at Flores Nabuco. From these algae it went readily onto bits of corn leaves and onion skin which had been added to the cultures. Another common species, *R. sphaerocarpum,* was found on *Oedogonium* sp., and *Mougeotia* sp. in a ditch at Saõ Carlos. Nothing new or different in structure and development was found in the Brazilian specimens of these species, so that further description of them is unnecessary.

In addition, two other *Rhizophydium* parasites of algae were found in Brazil which appear to be new. The first of these occurred on *Netrium* sp. at Flores Nabuco near Manaus. As shown in figure 23, it is distinguished by cone-shaped or slightly pyramidal sporangia with two to six narrow exit papillae and conical, hyaline, smooth resting spores. The sporangia are somewhat similar in shape to those of *R. verrucosum* (Cejp, 1933) but lack the warty wall and long tilted exit papilla. The shape of the sporangia and resting spores distinguishes this species from other known members of *Rhizophydium,* and it is accordingly regarded as a new species and named *R. conicum.*

RHIZOPHYDIUM **conicum** n. sp.—Fungus parasiticus; sporangiis sessilibus, hyalinis, levibus, conicis (14–16 × 20–28 μ), 2–6 papillatis; zoosporis sphaericis (2–2.5 μ); sporis perdurantibus hyalinis, levibus, conicis (6–8 × 10–14 μ), vel leviter irregularibus; modo germinationis ignoto.

Thalli numerous, up to 30 on a host. Sporangia hyaline, smooth, conical or pyramidal (14–16 × 20–28 μ) with two to six narrow exit papillae, 2 μ in diam. Zoospores spherical (2–2.5 μ), with a minute refractive globule; emerging from one or more of the lateral papillae. Rhizoidal system well developed and richly branched. Resting spores conical (6–8 × 10–14 μ), or slightly irregular, hyaline and smooth with one or more large refractive globules; germination unknown.

Parasitic on *Netrium* sp., Flores Nabuco, Manaus, Amazonas, Brazil.

The other species referred to above parasitized an unidentified species of *Closterium* from Flores Nabuco. It is characterized by spherical and broadly pyriform sporangia with a low, broad exit papilla (fig. 25) and light-brown, warty, thick-walled resting spores which lack large refractive globules (fig. 27). In addition the rhizoidal axis may be slightly inflated inside the host cell like a small apophysis as in *Phlyctochytrium.* The inflation is rarely more than 2 μ in diameter, and in some thalli it may be lacking. Therefore, this species is included in *Rhizophydium* instead of *Phlyctochytrium* for the time being. The exit orifice may be up to 8 μ in diameter, which often gives the empty sporangia an appearance similar to those of *R. globosum* and *R. carpophilum.* The warty, thick-walled resting spores resemble somewhat those of *R. asterosporum* and *R. goniosporum* (Scherffel, 1925), but the warts on the Brazilian species are more numerous and sometimes almost continuous. Furthermore, no attached companion or male cells were found on the resting spores. The author, therefore, regards this parasite as a distinct species and proposes the name *R. Closterii* for it.

RHIZOPHYDIUM **Closterii** n. sp.—Fungus parasiticus; sporangiis hyalinis, levibus, aut sphaericis (10–18 μ), aut pyriformibus (12–18 × 14–24 μ); l-papillatis; zoosporis sphaericis (2–2.5 μ); sporis perdurantibus sphaericis (10–16 μ), fuscis, verrucosis.

Thalli numerous, up to 18 on a host. Sporangia hyaline, smooth, spherical (10–18 μ), or broadly

elm pollen. ×1400.—Fig. 23, 24. Conical sporangia, resting spores, and zoospores of *R. conicum* on *Netrium* sp. ×1200.—Fig. 25, 26. Sporangia, resting spores, and zoospores of *R. Closterii* on *Closterium* sp. ×1500.—Fig. 27, 28. Resting spore of *R. Closterii* and germination of same. ×1600.—Fig. 29. *Rhizophydium* sp. on elm pollen. ×1400.—Fig. 30. Zoospores of same with greyish granular protoplasm. ×1600.—Fig. 31, 32. Thalli and zoospores of *R. Sphaerotheca* on elm pollen. ×1600.—Fig. 33. Early infection of a protozoan by *R. apiculum.* ×1200.—Fig. 33A. Zoospores of same. ×1600.—Fig. 34. Protozoan infected with 11 thalli of *R. apiculatum.* ×1200.—Fig. 35. Infected spore of protozoan. ×1200.—Fig. 36, 37. Germination of resting spores of *R. apiculatum.* ×1400.

pyriform (12–18 × 14–24 μ), with a low broad exit papilla, up to 8 μ in diam. Zoospores spherical (2–2.5 μ), with a minute refractive globule. Rhizoidal system fairly coarse, richly branched and sometimes extending to the full length of the host cell, usually inflated slightly at the base of the sporangium. Resting spores spherical (10–16 μ), with a thick (2–2.5 μ), warty, light-brown wall and coarsely granular content; functioning as prosporangia in germination.

Parasitic on *Closterium* sp. at Flores Nabuco near Manaus, Amazonas, Brazil.

Of the species isolated on fern spores and pollen grains *R. Sphaerotheca* (fig. 31, 32) and *R. pollinis-pini* were the most common. These species were isolated in water samples from Saõ Carlos, Matto Grosso, and Porto Velho and Manicore, Amazonas, which had been flown by plane to New York and baited with *Equisetum* spores and pollen grains. Within three weeks these cultures yielded an abundance of *R. Sphaerotheca* and *R. pollinis-pini*. The sporangia and zoospores of the Brazilian specimens agree closely in structure with those described by Zopf (1887) for these two species in Germany, and there is no reason for considering them to be different species.

In the same cultures on elm pollen another species was found which differs from all known members of *Rhizophydium* by its non-guttulate zoospores. Instead of containing a conspicuous refractive globule like those of other species, the zoospores have a coarsely granular, non-refringent content (fig. 30) which imparts to them and the sporangia (fig. 29) a grayish granular appearance. *Rhizophydium gibbosum* is the only other known species whose zoospores show any resemblance, but in this species the swarmspores include several small refringent globules. The presence or absence of refractive globules in the zoospores is usually a specific cell character of chytrids and has been used generally as a basis of species distinction. The author, however, questions the wisdom of using this character alone as a distinguishing mark, and for this reason the present species will be held in question until it is more fully known.

SUMMARY

Twelve species of *Rhizophydium* were isolated from water and moist soil samples collected at various places in the Amazon Valley in Brazil. Of these species seven are new. Three species, *R. hyperparasiticum* n. sp., *R. mycetophagum* n. sp., and *R. carpophilum*, respectively, are hyperparasites of chytrids, Zygomycetes and Oomycetes. *Rhizophydium Amoebae* n. sp., *R. apiculatum* n. sp., and *Rhizophydium* sp. parasitize microscopic animals; and *R. conicum* n. sp., *R. Closterii* n. sp., *Rhizophydium* sp., *R. globosum*, *R. Sphaerotheca*, and *R. pollinis-pini* attack algae, fern spores and pollen grains.

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THE NATURE OF CELLULOSE IN SPHAGNUM [1]

Nicolin Plank

ALMOST WITHOUT exception, the work which has been done in cellulose chemistry has been based upon the cellulose found in the higher plants. It may be incorrect to assume that the cellulose of lower plants is of the same type and is present in the same quantities as that of the higher plants. Therefore, the purpose of this paper is to report the results of a study of the cellulose of a Bryophyte, *Sphagnum recurvum* var. *tenuis* Kling collected from a peat bog, Chase Lake, located a few miles north of the Seattle city limits.

The term, cellulose, is here used to include not only the pure glucosan but also traces of mannan, xylan, and similar substances. Before undertaking quantitative studies, microchemical tests were carried out to confirm the presence and location of cellulose in peat moss tissues. A hydrocellulose reaction with IKI and a solubility test with cuprammonium hydroxide solution were used according to the method of Tunmann (1913) and Molisch (1913) and gave results indicating the presence of cellulose, although the color reactions were slightly different from those secured in similar tests on higher plants. These reactions indicated that the cell walls of *Sphagnum* were made up of cellulose or of a substance which reacted as cellulose does.

MATERIALS AND METHODS.—The amount of cellulose present in *Sphagnum* was determined by two methods, one volumetric and one gravimetric. For both methods, samples were prepared as follows: Several hundred grams of green, living *Sphagnum* were collected and separated completely from other plants and foreign materials. The moss was then laid on paper towels in the laboratory and air-dried at approximately 21°C. for a week. The moisture content of this air-dried material, determined by drying further at 100°C. in a weighing bottle and cooling in a desiccator, was found to be 1.3 per cent. *Sphagnum* is exceedingly hydroscopic, picking up water from the atmosphere so readily that for accurate analysis all samples had to be weighed in a stoppered bottle as rapidly as possible.

Volumetric procedure.—An accurately weighed sample of air-dried moss, prepared as above, was placed in a muslin bag in a Soxhlet funnel and extracted for four hours with 95 per cent ethyl alcohol to remove chlorophyll, waxes, fats, and other alcohol-soluble substances. Then it was air-dried and weighed again and the percentage dry weight remaining was computed. The residue was ground to pass a 0.5 mm. sieve and duplicate 0.1000 gm. samples of this powder were weighed out on watch glasses and transferred quantitatively to 250-ml. Erlenmeyer flasks. To each flask 100 ml. of 1 per cent NaOH solution was added with a pipette; the contents were mixed and then refluxed for two hours. The residue was filtered into a gooch, allowing it to form its own mat, and, after all the fibers were caught, washed with four 10 cc. portions of water at 60°C.

As much of the residue as possible was transferred to a 100 ml. beaker and 25 ml. of 12 M. sulfuric acid was added. The gooch was set in the beaker also to dissolve any of the moss that remained, and finally the whole solution was transferred quantitatively to a 250 ml. Erlenmeyer, using 50 cc. distilled water to help transfer each sample. Into each flask 25 ml. of 0.6 N. potassium dichromate solution was pipetted and the mixture refluxed for one hour, stoppered, and cooled. To this mixture, 40 ml. of distilled water and several drops of o-phenanthroline indicator (1.485 per cent o-phenanthroline monohydrate dissolved in 0.025 M. ferrous sulfate solution) were added and it was then titrated immediately with freshly prepared and standardized ferrous ammonium sulfate solution made up in 1 N. sulfuric acid, until the color change from green to pink occurred. The ferrous ammonium sulfate solution was standardized by titration against 10 ml. portions of 0.6 N. potassium dichromate solution in a flask containing 10 ml. of 95 per cent sulfuric acid, 90 ml. water, and 3 drops o-phenanthroline indicator.

From these data, the weight of cellulose present was computed, assuming that cellulose is $(C_6H_{10}O_5)_n$ and complete oxidation to carbon dioxide and water occurs. One milliliter of 1 N potassium dichromate is equivalent to 0.00675 gm. of cellulose. The above volumetric method is essentially that of James H. Kettering and Carl M. Conrad in their unpublished report to the Subcommittee on Cellulose Analysis; Cellulose Division of the American Chemical Society.

Gravimetric procedure.—About five grams of air-dried moss were weighed out and extracted by refluxing in a Soxhlet funnel with a 1:1 mixture of alcohol and benzene until the filtrate showed no more color (approximately four hours). The moss was again air-dried and weighed and it was found that approximately 30 per cent of its original weight had been lost.

Cross and Bevan cellulose was determined on duplicate samples, using a high grade cotton as a control. The extracted, accurately weighed samples were moistened with distilled water and allowed to form their own mat in a tared gooch, passing the filtrate through several times until all the fibers were caught. The gooch was then placed in the chlorination apparatus which consisted of an inverted funnel connected to the chlorine generator. The funnel fitted tightly over the gooch which was attached to a filter flask half filled with water and connected to an aspirator. The amount of chlorine passing

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through the sample in the gooch was controlled by changing the pressure in the aspirator and counting the number of bubbles rising from the end of the funnel projected below the surface of the water in the suction flask. Chlorine was passed through at the rate of two bubbles per second. Temperature was maintained at about 15°C.

After exactly three minutes of chlorination, the gooch was removed and washed by suction with distilled water and then 3 per cent sodium sulfite. The contents were transferred quantitatively to a 250 ml. beaker containing 100 ml. of freshly prepared 3 per cent sodium sulfite solution. This was covered with a watch glass and boiled for twenty minutes, then filtered back into the same gooch, allowing it to form its own mat again. After washing with ten 10 ml. portions of distilled water, the chlorination process was repeated. Alternate chlorinations and boilings were continued until the moss was completely white after chlorination and only slightly colored when sodium sulfite solution was added. Twelve chlorinations were necessary before the cellulose was sufficiently free of lignin.

After the last sodium sulfite treatment, the sample was washed with 300 ml. of hot distilled water, 50 ml. of 10 per cent acetic acid, 500 ml. of hot distilled water, and finally, 50 ml. of 95 per cent alcohol. The resulting total cellulose was dried at 100°C. for 12 hours, cooled in a desiccator, and weighed.

Determination of alpha, beta, and gamma celluloses.—After determination of total Cross and Bevan cellulose in *Sphagnum* by both gravimetric and volumetric methods, the resulting samples were analyzed for their content of the alpha, beta, and gamma celluloses. Samples of the Cross and Bevan cellulose were weighed out and analyzed for alpha cellulose by the method of the Technical Association of the Pulp and Paper Industry (1936). A similar sized sample of pure cotton was analyzed as a basis for comparison. All filtrate and wash water was saved for use in the subsequent beta and gamma cellulose determinations.

The alpha cellulose thus prepared was hydrolyzed quantitatively by the cellobiose octa-acetate method of Spencer (1929). A cotton sample was also treated in the same manner and the products obtained were definitely identified as cellobiose octa-acetate by melting point determinations.

To determine percentages of beta and gamma cellulose in the Cross and Bevan sample, the filtrate and wash waters from the alpha cellulose sample were put in a 500 ml. volumetric flask and diluted to volume. From this, 50 ml. portions were pipetted into Erlenmeyer flasks, treated with 10 ml. of standard (1.835 N.) potassium dichromate solution and 100 ml. of 72 per cent sulfuric acid and boiled for five minutes. After cooling, the excess potassium dichromate was titrated with freshly prepared and standardized ferrous ammonium sulfate solution, using o-phenanthroline as an indicator. From the results of this titration, the amount of beta and gamma cellulose that was oxidized could be calculated. To find out the percentage of this that was gamma cellulose, another 50 ml. sample was neutralized first with sulfuric acid to precipitate the beta cellulose. The precipitate was filtered on a fine sintered glass gooch, washed, and the filtrate and washings treated with potassium dichromate and sulfuric acid as above. Titration of this solution gave the percentage of gamma cellulose present, and beta cellulose content could then be estimated. The above procedure is modified slightly from that recommended by Charles Doree (1933).

RESULTS AND DISCUSSION.—All samples in the foregoing processes were run in duplicate or triplicate until checks well within the range of unavoidable experimental error were obtained. The following values represent an average of the samples run:

TABLE 1. *Results from the volumetric procedure for estimating total cellulose content.*

Per cent loss in weight by extraction	12.74%
Weight of extracted sample	0.1249 gm.
Volume $K_2Cr_2O_7$ added (0.6 N.)	25.00 ml.
Volume $K_2Cr_2O_7$ reduced (0.6 N.)	15.59 ml.
Grams of cellulose oxidized	0.0632 gm.
Per cent cellulose in extracted sample	50.60%
Per cent cellulose in original moss	44.16%

TABLE 2. *Results from the gravimetric procedure for estimating total cellulose content.*

Per cent loss by extraction	29.91%
Weight of extracted sample	1.7364 gm.
Weight of Cross and Bevan cellulose	1.1609 gm.
Per cent Cross and Bevan in extracted moss	66.86%
Per cent Cross and Bevan in original moss	46.69%

TABLE 3. *Results of alpha, beta and gamma cellulose and cellobiose octa-acetate preparation.*

	Moss	Cotton
Per cent α-cellulose in C. and B. sample	43.43%	98.69%
Per cent β-cellulose in C. and B. sample	15.05%	.....
Per cent γ-cellulose in C. and B. sample	32.94%	.....
Per cent yield of cellobiose octa-acetate	3.8%	35.4%
Melting point of octa-acetate	220°C.	221°C.

TABLE 4. *Percentage composition of Sphagnum.*

Percent alcohol-benzene extractives, lignin, water	57.16%
Per cent α-cellulose	20.28%
Per cent β-cellulose	7.07%
Per cent γ-cellulose	15.49%

The cotton sample run simultaneously with the above moss sample gave 96.23 per cent Cross and Bevan cellulose, showing that hydrolysis was not extensive.

These two percentages (44.16 and 46.69 per cent) of total cellulose content in the original samples of *Sphagnum* are close enough together to be considered as checks in two processes which are so widely dif-

ferent and involve such a great number of opportunities for error. The higher percentage of Cross and Bevan cellulose where a lower one would be normally expected because of hydrolysis is accounted for in that the final Cross and Bevan cellulose obtained was not pure but still contained enough lignin to give it a brownish color when dry. Further chlorinations and boilings had little effect on purifying this and only caused hydrolysis. Therefore, the lower figure obtained from the volumetric determination is probably more nearly correct.

These percentages of the three types of cellulose total only 91.42 per cent of the Cross and Bevan cellulose, apparently indicating that over 8 per cent was made up of material other than cellulose. The alpha, beta and gamma cellulose percentages in the original moss, calculated from the above data, total 42.84 per cent. This value, averaged with the volumetric value of 44.16 per cent is probably an acceptable value for the true percentage of total cellulose.

From the above results, it can be stated that approximately 43 per cent of air-dried *Sphagnum recurvum* var. *tenuis* Kling is cellulose. It is interesting to compare this finding with the report of Shikata and Watanabe (1934) which gives 48.53 per cent cellulose for a species of *Sphagnum* native to Japan. The difficulties encountered in securing accurate values by use of the customary cellulose analytical techniques were many and most of these methods had to be modified before they could be employed with peat moss. Most of the problems arose because of the hygroscopic properties of the moss and moss cellulose, and because acids and alkalies, even in small concentrations, tended to make the cellulose gelatinous and impossible to filter. When dry, crude *Sphagnum* cellulose is very hard and, unlike the usual wood cellulose, is of a grayish or brownish color.

The apparent ease with which *Sphagnum* cellulose hydrolyzes in the presence of acids or bases indicates that there exists in this material a different sort of linkage between sugar units than exists in higher plant cellulose. Another interpretation of the low alpha cellulose yield may be that there exists a relatively low molecular weight cellulose originally present in the sample, and successive chlorinations bring about further hydrolysis and oxidation to such an extent that the degree of polymerization falls below that represented by the alpha cellulose group. The formation of carboxyl groups during chlorination would also contribute to the solubility in 17½ per cent sodium hydroxide. Alpha cellulose makes up only about 43 per cent of the total cellulose content, compared with pure cotton which is 98 to 99 per cent alpha cellulose. The rest of the cellulose present is in beta and gamma forms, presumably comprising short chains of glucose anhydride units and, in addition, probably polymers of other sugars such as xylans, and oxidation products such as oxycellulose. This is an unusually high value of beta and gamma forms as compared with values based on cellulose from the higher plants.

The very low percentages of cellobiose octa-acetate obtained seem to indicate that, while alpha cellulose may have been present in the original sample, a considerable portion of the glucose residues were probably oxidized, and thus precluded from contributing to formation of the cellobiose octa-acetate. It is also possible that the original cellulose may have been high in sugar polymers which were not glucosans. At least 3.8 per cent of the carbohydrates present are definitely glucosans and are linked together by the beta 1,4 glycosidic linkage.

SUMMARY

Cross and Bevan cellulose was prepared from *Sphagnum recurvum* var. *tenuis* Kling and the total amount of cellulose was also determined by a volumetric method. From the crude cellulose, alpha cellulose was prepared and the amounts of beta and gamma celluloses determined by the process outlined. The results indicated that peat moss is lower in the long-chain and pure glucose anhydride cellulose than samples of Spermatophyte plant tissues which have been similarly investigated. Results of the cellobiose octa-acetate preparation from the alpha cellulose obtained indicate that at least 3.8 per cent of the carbohydrates present in *Sphagnum* cellulose are definitely glucosans and are linked together by the beta 1,4 glycosidic linkage.

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ARTIFICIAL AND NATURAL HYBRIDS IN THE GRAMINEAE, TRIBE HORDEAE I. ELYMUS, SITANION, AND AGROPYRON [1]

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THE OCCURRENCE of natural hybrids in the grass family has been noted by many authors, principally in Europe. Cugnac (1937) has pointed out that such hybrids are to be expected with greater frequency in the Gramineae than in other families because species of this family most often occur in large stands, and not infrequently two or more related species are closely intermingled. Furthermore, their habit of wind pollination increases the chances of cross pollination, both within and between species.

These hybrids are important from both the purely scientific and the practical viewpoint. From the standpoint of the systematics of the Gramineae, it is important to note that a number of forms originally described as species, such as *Festuca loliacea* Curt., and *Oryzopsis Bloomeri* (Boland.) Ricker have later been demonstrated to represent collections of sterile F_1 hybrids (Jenkin, 1933; Johnson, 1945; Johnson and Rogler, 1943). There is every reason to believe that other so-called "species" consist likewise of hybrids, and the evidence presented in this and succeeding papers indicates that this is true of certain "species" described in the tribe Hordeae. It is also noteworthy that most of the hybrids to be described below, like the two just mentioned, are between species which according to the traditional system of classification belong in different genera. The frequent occurrence of such "intergeneric" hybrids and the ease with which many of them can be produced artificially suggest that a drastic revision of this and other tribes of the grass family may eventually be necessary.

From the standpoint of the phylogeny and evolution of the Gramineae, these hybrids are important as the possible ancestors of new species and as indicators of the method by which many of the present-day species have originated. The origin of new plant species by means of hybridization and allopolyploidy is now widely recognized, and the actual parentage of many natural allopolyploids, including species of grasses, is now known (Clausen, Keck and Hiesey, 1945). Since polyploidy is particularly common in the Gramineae, reticulate evolution by this method has probably played a larger part in the development and differentiation into species within the grass family than in most other families of Angiosperms.

The practical importance of these hybrids centers about the attempts of the senior author to obtain new allopolyploid species of grasses which will be of value in revegetating depleted stock ranges. The number of hybrid combinations possible even within a relatively small group of species of Gramineae is so large that some indication of the most vigorous and potentially successful combinations is desirable. This is amply provided by the existence of sterile hybrids growing beside their parents in natural habitats, often in considerable numbers, and forming more vigorous clumps than their fertile parents. It seems likely that such hybrids, if they can be rendered fertile by doubling their chromosome number artificially through the agency of colchicine, will give rise to particularly vigorous and well adapted new species.

The identification of natural hybrids can be made with considerable certainty by means of careful field studies and even by examination of herbarium specimens, as has recently been brilliantly demonstrated by Johnson (Johnson and Rogler, 1943; Johnson, 1945). Nevertheless the final proof of the hybrid nature of a plant is its artificial reproduction through controlled emasculation and cross pollination. For this reason, the resemblance between the natural hybrids described in this paper and the corresponding artificial ones is of particular interest, and it seems justifiable to group them together in the discussion of their morphological and cytological characteristics.

MATERIAL AND METHODS.—For obtaining the artificial hybrids, the same technique was adopted as had previously been successful in *Bromus* (Stebbins and Tobgy, 1944), and the cytological methods, including fixation of material, making of smear preparations and observation and recording of results are as described in that paper. The chromosome drawings are reproduced at a magnification of 1500×. In those natural hybrids which were identified from herbarium specimens, pollen was examined by boiling florets in water, dissecting out the anthers, crushing them on a microscopic slide in a solution of cotton blue in lactic phenol and covering them with a cover glass. Under these conditions, the fertile pollen grains of the parent species become heavily stained in an hour or so, while those of the sterile hybrids remain clear and transparent. In addition, the pollen grains of the hybrids are smaller and often irregular in size, as has frequently been observed in hybrids.

Both the parents of the artificial hybrids and the living material of the natural hybrids were collected by the senior author in the field, except for a few collections which were obtained from the U. S. Soil Conservation Service and from the Carnegie Institution of Washington laboratory at Stanford University. To the directors of this institution the authors give many thanks for this material. The exact source of each culture will be given below with the description of the hybrids.

ORIGIN AND DESCRIPTION OF THE HYBRIDS.—1. *Artificial hybrids.* From a single pollination of 40 emasculated florets of *Elymus glaucus* with *Sitanion jubatum,* no hybrid seeds were obtained. This, however, is insufficient evidence on which to judge the

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TABLE 1. *Morphological characteristics of the species and hybrids.*

Species or hybrid	Source of material	Maximum length of spike (cm)	Mean length of internode (cm)	Number of spikelets per node	Fragility of rachis	Length of glumes (including awns, mm)	Division of glumes	Length awn of lemma (mm)	Length anthers (mm)
Species:									
Agropyron Parishii Scribn. and Smith	Hastings Res., 3432	26	1.8	1(-2)	cont.	9	entire	7	5.5
Agropyron pauciflorum (Schwein.) Hitchc.	Leesville, 2957, 403	15	0.85	1	cont.	12	entire	5	2.0
Agropyron sp., "San Benito"	San Benito Co., 2776, 401	21	1.2	1(-2)	cont.	11	entire	1.5	5.5
Elymus glaucus Buckl.	Hastings Res., 3445	18	0.75	2	cont.	17	entire	21	2.0
E. glaucus v. Jepsoni Davy	Payne's Creek, 2948, 405	16	0.7	2	cont.	16	entire	31	4.2
Sitanion hystrix (Nutt.) J.G.Sm.	Walker Pass 2849, 401	7	0.35	2	frag.	36	1–2–fid	30	1.6
Sitanion jubatum	Tiburon, Howell 18072	8	0.45	2	frag.	80	2–5–fid	65	1.8
Sitanion jubatum J. G. Smith	Hastings Res. (7 specimens)	9	0.45	2	frag.	85	2–5–fid	69	1.8
Artificial hybrids:									
A. "San Benito" × A. pauciflorum	548–3	23	0.9	1(-2)	cont.	12	entire	1.5	2.7
A. "San Benito" × E. glaucus var. Jepsoni	549–2	14	0.9	1–2	cont.	11	entire	8	2.7
A. "San Benito" × S. hystrix	553–2	18	0.65	1–2	part frag.	19	1–2–fid	12	2.5
A. pauciflorum × E. glaucus var. Jepsoni	550–2	11	0.6	1–2	cont.	11	entire	8	2.2
Natural hybrids:									
A. Parishii × S. jubatum	Hastings Res., 3429	19	1.0	1–2	part frag.	24	1–2–fid	34	2.8
A. pauciflorum × E. glaucus	Mather, CI–4330	29	1.3	1–2	cont.	12	entire	13	2.4
A. pauciflorum × S. jubatum	Richmond, 3388	6	0.65	1	part frag.	27	1–2–fid	31	2.0
E. glaucus × S. jubatum	Hastings Res., 3427	13	0.6	2	part frag.	34	2–3–fid	41	1.8
E. glaucus × S. jubatum	Tiburon, no. 3360	9	0.4	2	part frag.	31	1–2–fid	34	2.0

ease of crossing between these two species, since undoubted hybrids between them are rather common in nature. The hybrid between *Agropyron* and *Sitanion* was obtained once, using as the pistillate parent a strain originally identified as *A. pauciflorum,* and collected near San Benito, Calif. (herb. spec. no. 2776). It is a tall, loosely growing type, with an elongate spike, short or obsolete awns and larger anthers than is characteristic for *A. pauciflorum.* The cytological evidence presented below, along with its somewhat anomalous morphological characteristics and its distribution outside of the normal range of *A. pauciflorum* in California, indicates that it does not belong with that species. It is closer perhaps to *A. Parishii,* although differing from typical plants of the latter species in its green rather than glaucous character, its less elongate spikes with a stouter, stiffer rachis, its shorter awns and its less prominently exserted anthers. For the present, this anomalous *Agropyron* will be referred to simply as "San Benito." Forty florets of one spike of "San Benito" were emasculated and pollinated by a plant of *Sitanion hystrix* grown from seed collected at the summit of Walker Pass, Kern County (no. 2849). Two seeds were obtained, both of which produced vigorous plants, intermediate in both vegetative and floral characteristics between their parents. The hybrids typically produce a large number of sterile shoots and relatively few culms. They have the dense, caespitose habit characteristic of *S. hystrix,* but with more elongate leaves, although these are by no means as long as those of the *Agropyron* parent. The spike is intermediate in length between those of the two parents, is rather densely flowered and has the spikelets mostly two at a node, as in *Sitanion.* Because of these features, the flowering spike of this hybrid bears a superficial resemblance to that of *Elymus glaucus,* but may at once be distinguished from that species by its glumes, which have a relatively short body in relation to the length of the awn, and are frequently bifurcate, as in *Sitanion* (fig. 2, B). The principal diagnostic characters of this and other hybrids are tabulated in table 1.

Three different types of hybrids were obtained involving *Agropyron* and *Elymus glaucus.* Two of

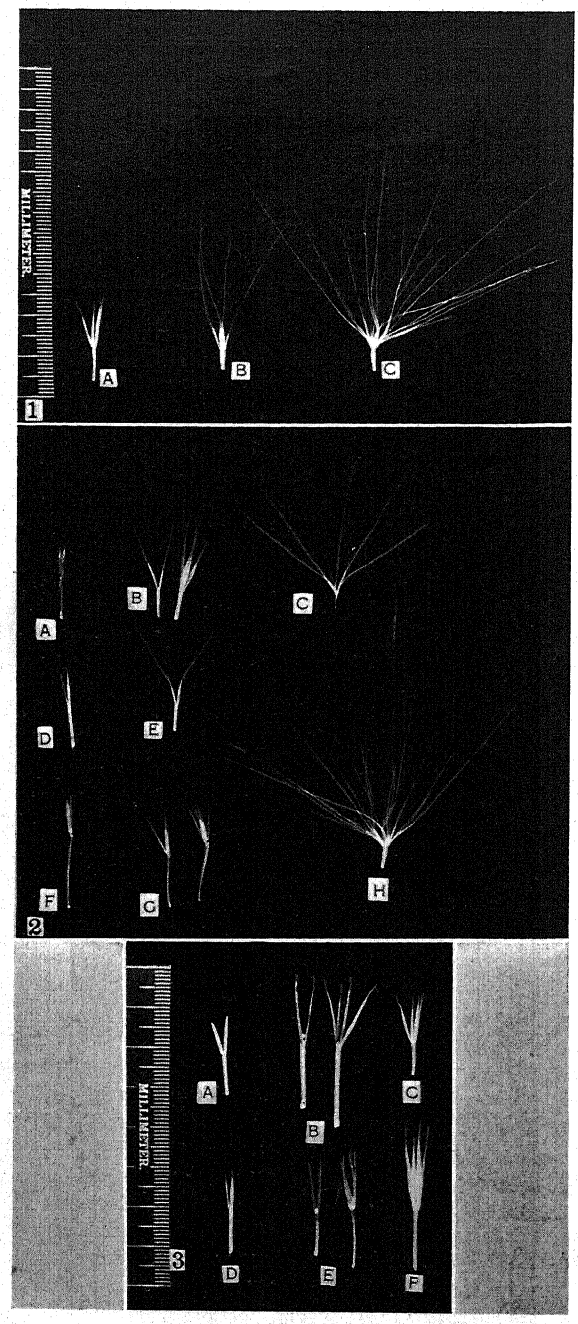


Fig. 1–3. Internodes of rachis and glumes, ×⅔.—Fig. 1. A, *Elymus glaucus;* B, *E. glaucus* × *S. jubatum;* C, *Sitanion jubatum.*—Fig. 2. A, *Agropyron pauciflorum;* B, *A.* sp. "San Benito" × *S. hystrix;* C, *Sitanion hystrix;* D, *Agropyron* sp., "San Benito"; E, *A. pauciflorum* × *S. jubatum ;* F, *A. Parishii;* G, *A. Parishii* × *S. jubatum;* H, *Sitanion jubatum.*—Fig. 3. A, *Agropyron pauciflorum;* B, *A. pauciflorum* × *E. glaucus,* no. CI4330; C, *Elymus glaucus;* D, *Agropyron* sp., "San Benito"; E, *A.* sp., "San Benito" × *E. glaucus* var. *Jepsonii;* F, *Elymus glaucus* var. *Jepsonii.*

them were from an emasculated spike of the San Benito strain, of which forty florets were pollinated by a plant of *Elymus glaucus* Buckl. var. *Jepsoni* Davy grown from seed collected at Payne's Creek, Tehama County (no. 2948). The twelve seeds obtained from this pollination (culture no. 549) all grew into vigorous plants, intermediate between *Agropyron* and *Elymus,* and completely sterile. Eleven of these are similar to each other in appearance and show some of the pubescence characteristic of the *Elymus glaucus* var. *Jepsoni* parent, particularly on the upper surface of the leaves. Their spikes are relatively short and densely flowered, with the spikelets mostly two at the nodes (fig. 3, B).

Another *Agropyron* × *Elymus* hybrid resulted from exposing to pollen of the same *E. glaucus* var. *Jepsoni* plant a spike of a plant of *A. pauciflorum* grown from seed collected at Leesville, Colusa County (no. 2957). This strain is densely caespitose, with pale green, rather narrow leaves; moderately loosely flowered spikes; short, practically awnless glumes and lemmas; and small anthers. From this spike five seeds were obtained, four of which germinated (culture no. 550). Two of the seedlings were selfs, and the other two were hybrids, with the pubescence of the *Elymus* parent expressed as in the no. 549 hybrids. From these they differ in their smaller size, paler, shorter leaves, in bearing most often a single spikelet at each node of the spike (fig. 3, E), and in their smaller anthers. These also are completely sterile as to both pollen and seed setting.

2. *Natural hybrids.* The natural hybrid found most commonly by the writers is that between *Elymus glaucus* and *Sitanion jubatum.* The first plant recognized as this hybrid was found at Tiburon, Marin County, in June, 1943 (no. 3360) where the hybrid was growing with its parents. This clone has been growing vigorously in Berkeley for two years, often several different divisions of it. No seed has yet been found on it, although thousands of florets have been examined and although plants of both parents grown under identical conditions set 90 to 100 per cent of plump, viable seed. The only fertility yet seen in no. 3360 was from plants treated with colchicine, and the seedlings from these fertile spikes yielded, as expected, allopolyploids with 56 chromosomes. Morphologically, it is exactly intermediate between its putative parents. It is intermediate in height, is moderately pubescent on the leaves, but not densely so as is the strain of *S. jubatum* growing with it, while the spike has a partly fragile rachis. The glumes are somewhat divided into awn-like segments, as in *Sitanion,* but their basal part is relatively broad and flat, as in *Elymus* (fig. 1, B). The intermediate characteristics of this plant are more completely shown in table 1. Another collection of this same hybrid, along with its parents, was sent to the senior author in 1943 by Mr. Paul Dickey of the U. S. Soil Conservation Service nursery at Pleasanton. This was collected in the Ukiah Valley, Mendocino County.

In 1944, similar plants were seen in abundance

on the Frances Simes Hastings Natural History Reservation, near Jamesburg, Monterey County, where again they were always associated with *Elymus glaucus* and *Sitanion jubatum*. These were likewise completely sterile, but seeds have again been produced from colchicine-treated plants. The plants collected at the Hastings Reservation by its director, Dr. J. M. Linsdale, had been identified as *Sitanion Hanseni*. The senior author, therefore, reexamined his herbarium specimens and living cultures of *S. Hanseni*, including those of which the chromosome number had been reported (Stebbins and Love, 1941), and found that all agreed with the *Elymus glaucus* × *Sitanion jubatum* hybrids, and all were likewise completely sterile as to both pollen and seed setting. In addition 27 specimens of *S. Hanseni* were examined from the following herbaria: University of California, California Academy of Sciences, U. S. National Herbarium, University of Idaho, Southern Branch. All of these agreed morphologically with the hybrids collected by the authors, grown under culture and studied cytologically, and like them all had completely sterile pollen, while the caryopses were consistently empty. In 1945 the senior author found growing in the vicinity of Mather, Tuolumne County, similar sterile plants along with *Elymus glaucus* and *Sitanion hystrix*, and obviously intermediate between the plants of these two species with which they were growing. These apparent hybrids between *E. glaucus* and *S. hystrix* differ from the *E. glaucus* × *S. jubatum* hybrids only in that the glumes, although long awned, are not or only very rarely divided into awn-like segments. Both would be classified as *S. Hanseni*, so that this "species" actually consists of a series of sterile F_1 hybrids between *Elymus glaucus* and either *Sitanion jubatum* or *S. hystrix*.

The hybrid between *Agropyron pauciflorum* and *Sitanion jubatum* no. 3388, was found in 1943 on the hills of Contra Costa County about 6 miles north of Berkeley, opposite the Mira Vista Country Club. Both parents were abundant in the same site. In culture, it has shown the same vigor and sterility as the *Elymus-Sitanion* hybrids, but as yet no seed has been obtained from divisions treated with colchicine.

In habit, this hybrid is smaller than those between *Elymus* and *Sitanion*, and has narrower leaves, reflecting those of its *Agropyron* parent. The spikes are relatively short, and bear almost exclusively a single spikelet at each node of the rachis. The rachis is somewhat fragile, while the glumes and lemmas are much like those of the *Elymus-Sitanion* hybrids (fig. 2, E).

This hybrid corresponds to the description of *Agropyron Saundersii* (Vasey) Hitchc., except that it is smaller in stature and has shorter, more slender awns. The type specimen of *A. Saundersii*, loaned through the kind permission of Mrs. Agnes Chase from the U. S. National Herbarium, is a robust plant agreeing in its diagnostic characteristics with the hybrid no. 3388, except that the lateral awns on the glumes are less strongly developed. This would be expected from the fact that the *Sitanion* collected by Vasey in the same locality as *A. Saundersii* is a form of *S. hystrix* which has long, entire or merely bifid glumes. Since the type of *A. Saundersii* has the pollen grains completely empty, and shows no evidence of forming caryopses, it is probably an F_1 hybrid between *Agropyron pauciflorum* and *Sitanion hystrix*. The remaining 14 collections of *A. Saundersii* seen by the senior writer in the U. S. National Herbarium, and those of the University of California, California Academy of Sciences, and the Missouri Botanical Garden, are likewise without good pollen or caryopses. Their external morphology and the localities of collection indicate that they are hybrids between *A. pauciflorum* and *S. hystrix*. Dr. J. T. Howell, of the California Academy of Sciences, informed the writers (oral comm.) that in the case of the fine collections of *A. Saundersii* made by him in Tuolumne Meadows and vicinity, Yosemite National Park, both *A. pauciflorum* and *S. hystrix* were growing abundantly in the same site. So far as we are aware, our no. 3388 is the only collection of *A. pauciflorum* × *S. jubatum* that has been made to date.

The hybrid *Agropyron Parishii* × *Sitanion jubatum* has been found abundantly on the Hastings Reservation, always in the immediate vicinity of its parents. It differs from no. 3388 in exactly the same characters that separate *A. Parishii* from *A. pauciflorum*, namely, taller culms, less caespitose habit, more elongate spikes with longer internodes, and longer anthers. The spikelets are usually in pairs at the central nodes of the spike, but are always single on the upper nodes, and usually so on the lower (fig. 2, G). This difference from no. 3388 might be expected from the fact that in *A. Parishii* occasional spikes bear two spikelets at the central two or three nodes. Specimens exactly corresponding to our no. 3429 and others from the Hastings Reservation were collected by J. Burtt Davy about thirty miles west of our locality in the vicinity of Pico Blanco, where *A. Parishii* was also collected. In addition, the senior author has collected it on the mountain ridges south of Big Sur Canyon, about ten miles south of Davy's locality, again growing with *A. Parishii* and *S. Jubatum*. As with other natural hybrids grown in culture, fertile seeds were obtained only after treating the plants with colchicine.

The natural hybrid between *Agropyron pauciflorum* and *Elymus glaucus*, Carnegie Institution no. 4330, agrees in all of its characteristics with the artificial hybrid culture no. 550, except that it is larger and more robust throughout, and has broader leaves. This corresponds with the differences between the strain of *A. pauciflorum* found at Leesville, and those growing at Mather. C. I. no. 4330 was collected by Dr. W. M. Hiesey in 1943 with *A. pauciflorum* and *E. glaucus* in the meadow beside the Institution's experimental garden at Mather,

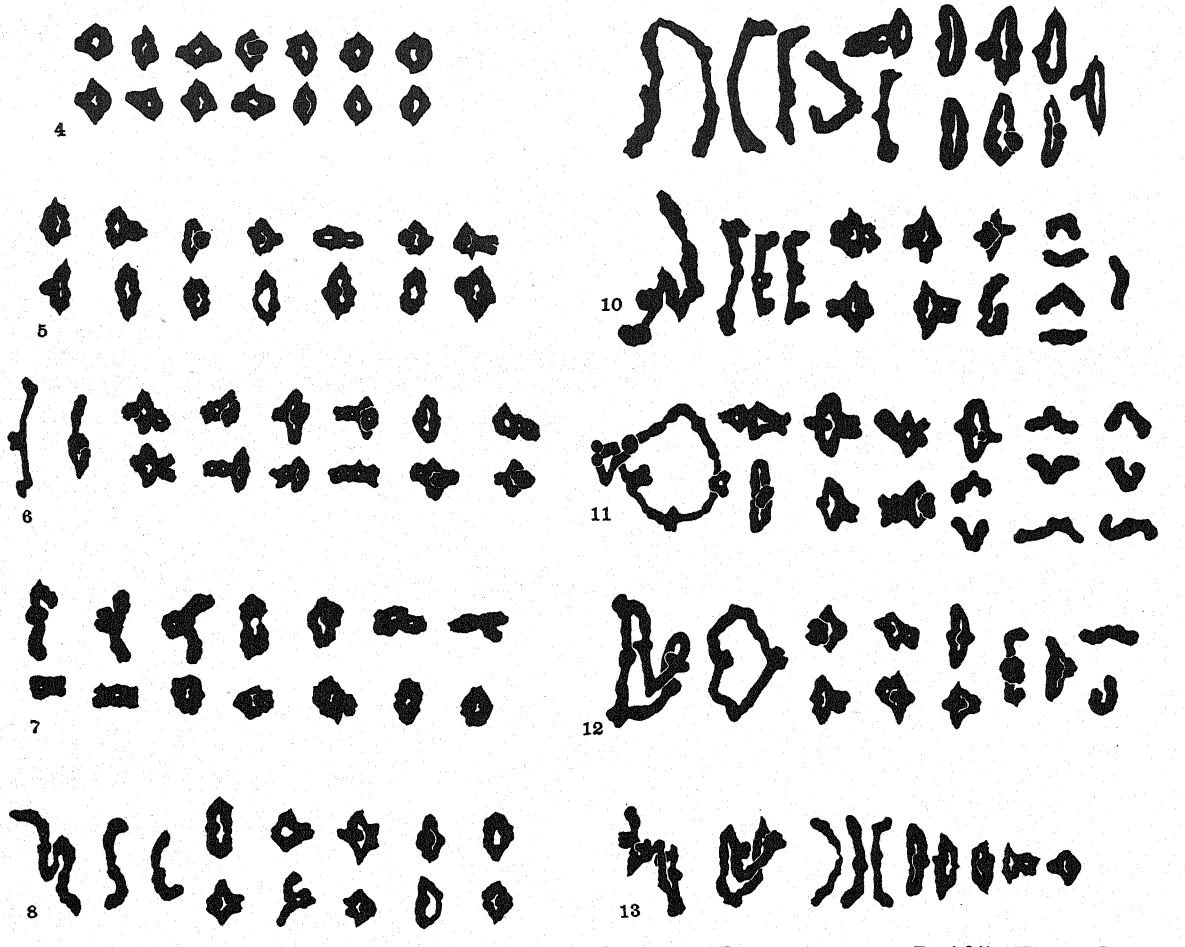


Fig. 4–13.—Chromosome configurations at I metaphase, all ×1500.—Fig. 4. *Agropyron Parishii.*—Fig. 5. *Elymus glaucus.*—Fig. 6. *Sitanion jubatum.*—Fig. 7. *E. glaucus* × *S. jubatum.*—Fig. 8. *A. Parishii* × *S. jubatum.*—Fig. 9. *A.* sp., "San Benito" × *S. hystrix.*—Fig. 10. *A. pauciflorum* × *S. jubatum.*—Fig. 11. *A.* "San Benito" × *A. pauciflorum.*—Fig. 12. *A. pauciflorum* × *E. glaucus,* no. 550–2.—Fig. 13. *A. pauciflorum* × *E. glaucus,* no. CI4330.

Tuolumne County. The senior author visited this locality in 1945 and found a large number of hybrid plants growing in a dense stand with their parents. It is noteworthy that many spikes of this hybrid have the spikelets single at every node, so that specimens bearing only such spikes would unhesitatingly be identified by any systematist as *Agropyron,* and would fall entirely within the description of *A. subsecundum* (Link.) Hitchc.

CHROMOSOME BEHAVIOR.—*Parents.*—As recorded in previous literature (cf. Stebbins and Love, 1941), all of the species which formed the parents of the hybrids described here have the somatic chromosome number $2n = 28$. The only species for which the number 28 is reported for the first time is *Agropyron Parishii* Scribn. and Smith, the count being based on meiosis in a plant collected on the Hastings Reservation, near Jamesburg, Monterey County (G. L. Stebbins, Jr., no. 3432). The species also agree in the size and morphology of their chromosomes to such an extent that chromosomes from different species cannot be distinguished from each other even when present in the same hybrid nucleus. There are slight differences in size between the different chromosomes of the same set, but these are too small to permit the certain identification of any particular chromosome from one nucleus to the next. Examination of well spread chromosome groups at first anaphase indicates that twelve of the fourteen chromosomes in the haploid set of each species have median or nearly median fiber attachment constrictions, while two have submedian ones, with a ratio between the two arms of 1:2 to 1:3.

At meiosis, fourteen bivalents are regularly formed in all of the species. The number and distribution of chiasmata, however, is markedly different, so that the species are of three kinds with respect to this character. The first is *Agropyron Parishii,* in which the great majority of the bivalents are simple rings, with one chiasma in each arm, and in which the mean number of chiasmata per nucleus is 28 (fig. 4 and table 2). In *Elymus glaucus* and *Agropyron pauciflorum* bivalents with one chiasma in each arm are still the most common type, but other types, particularly those with three chiasmata, are much more evident. Bivalents with

TABLE 2. *Chromosome behavior at first metaphase.*

Species or hybrid	Plant number	No. cells	Chromosome associations: Mean number per cell VI	V	IV	III	II	I	No. cells	Mean no. xta per cell	Chiasma frequencies: Mean no. ½ xta per chromosome	½ xta per chromosome in: VI	V	IV	III	II
Species:																
Agropyron Parishii	3432	77	0	0	0	0	14	0	35	28.20	2.01	0	0	0	0	2.01
Agropyron pauciflorum	403–4	50	0	0	0	0	14	0	15	30.00	2.14	0	0	0	0	2.14
Elymus glaucus	404–2	50	0	0	0	0	14	0	12	31.00	2.21	0	0	0	0	2.21
Sitanion jubatum	402–1	63	0	0	0	0	13.97	.06	11	39.00	2.79	0	0	0	0	2.79
Artificial hybrids:																
A. "San Benito" × A. pauciflorum	548–3	29	.34	.34	.28	.21	10.10	1.44	29	21.10	1.53	1.73	1.60	1.56	1.33	1.63
A. "San Benito" × E. glaucus	549–2	30	0	0	.10	0	12.86	3.46	6	26.30	1.88	0	0	1.50	0	2.13
A. "San Benito" × S. hystrix	553–2	32	0	0	.06	0	12.72	4.62	22	22.81	1.62	0	0	1.50	0	1.77
A. pauciflorum × E. glaucus	550–2	27	.48	.04	.40	.33	10.40	1.55	27	26.06	1.86	1.92	2.00	1.77	1.60	2.00
Natural hybrids:																
A. Parishii × S. jubatum	3429	27	0	0	.59	.15	11.81	1.55	18	22.60	1.61	0	0	1.60	1.33	1.74
A. pauciflorum × E. glaucus	C14330	42	.24	.10	.71	.69	9.64	1.89	42	24.38	1.74	1.80	1.60	1.58	1.33	1.98
A. pauciflorum × S. jubatum	3388	34	.12	.12	.29	.50	10.20	3.62	34	18.70	1.33	1.67	1.60	1.55	1.33	1.54
E. glaucus × S. jubatum	3360	67	0	0	0	0	13.81	.38	22	29.00	2.07	0	0	0	0	2.07

one or two chiasmata confined to a single chromosome arm are also more frequent. In *E. glaucus* 9.3 per cent of the bivalents are paired in one arm only, as compared with 4 per cent in *A. Parishii,* in spite of the fact that the total number of chiasmata per nucleus is greater in *E. glaucus,* this figure being 31 (fig. 5 and table 2). The third type, containing *Sitanion jubatum* and probably *S. hystrix,* is characterized by a considerably higher chiasma frequency, the mean number of chiasmata being 39 per nucleus, but at the same time the number of bivalents with chiasmata in one arm only is the same as in *E. glaucus,* namely, 9 per cent (fig. 6 and table 2). The percentage of arms with two or three chiasmata is correspondingly greater. The situation is summarized in table 3. This table shows that the chiasma frequency is the most variable in *S. jubatum,* a fact emphasized by the occurrence of one sporocyte out of 53 with 13 bivalents and 2 univalents.

TABLE 3. *Distribution of chiasmata per chromosome arm.*

	Percentage of arms with: 0 xta	1 xta	2–3 xta
Agropyron Parishii	2%	97%	1%
Elymus glaucus	4.7%	82.9%	12.4%
Sitanion jubatum	4.5%	60.2%	35.3%

The material was unfavorable for observation of midprophase stages, so it is not certain whether these differences in frequency and distribution of chiasmata observed at metaphase exist also at the time of chiasma formation. However, observation of a few sporocytes of *A. Parishii* at early to mid-diakinesis indicates that it is nearly the same at these stages as it is at metaphase. It is most likely, therefore, that the observations recorded reflect differences in the incidence of chiasmata at least to a large extent.

In most of the material of the parental species meiosis was regular, with only exceptional irregularities at later stages (table 4). Lagging chromosomes and chromatids were found in 1.3 per cent of the sporocytes at first anaphase in *E. glaucus* and in 3.8 per cent of the sporocytes in *S. jubatum* (table 4). None was observed in 25 sporocytes of one plant (no. 403–4) of *A. pauciflorum,* while in *A. Parishii* these stages were not available for observation. Bridge fragment configurations occurred in the species to a similar extent (table 4). The situation at second anaphase was essentially similar, except that *E. glaucus* had a somewhat higher percentage of lagging (12.9 per cent), and bridge-fragment configurations were absent in all cases except in the plant discussed below. The percentage of quartets with micronuclei was in the range of expectation on the basis of observed irregularities in meiosis.

In one plant of *A. pauciflorum,* no. 403–1, the percentage of meiotic abnormalities was distinctly higher. At first anaphase, 12 per cent of the sporo-

TABLE 4. *Chromosome behavior at later meiotic stages.*

Species or hybrid	Plant number	Anaphase I: No. cells	Anaphase I: Per cent with lag-gards	Anaphase I: Per cent with bridge-frag-ments	Anaphase II: No. cells	Anaphase II: Per cent with lag-gards	Anaphase II: Per cent with bridge-frag-ments	Tetrads: No. cells	Tetrads: Per cent with micro-nuclei
Species:									
Agropyron Parishii	3432	...	...	...	126	0.8	4.0	170	0.6
Agropyron pauciflorum ..	403–4	25	0	0	67	5.9	0	50	0
Agropyron pauciflorum ..	403–1	107	12.0	0	58	17.0	7.0	189	17.0
Elymus glaucus	404–2	77	1.3	0	38	12.9	0	112	6.2
Sitanion jubatum.....	402–1	79	3.8	3.8	65	1.5	0	151	3.9
Artificial hybrids:									
A. "San Benito" × A. pauciflorum	548–3	143	75.5	9.3	...	...	...	94	53.2
A. "San Benito" × E. glaucus	549–2	...	...	...	53	98.1	3.8	50	78.0
A. "San Benito" × S. hystrix	553–2	244	41.3	3.7	116	71.6	0.4	502	54.9
A. pauciflorum × E. glaucus	550–2	74	28.3	1.3	100	69.0	4.0	100	49.0
Natural hybrids:									
A. Parishii × S. jubatum	3429	62	23.0	0	171	46.8	0	194	38.7
A. pauciflorum × E. glaucus	CI4330	64	26.5	15.6	100	75.0	4.0	171	57.0
A. pauciflorum × S. jubatum	3388	56	54.0	5.0	106	91.0	4.7	165	72.0
E. glaucus × S. jubatum	3360	67	10.4	2.9	92	21.7	0	142	17.6

cytes had laggards. At second anaphase, lagging univalents were found in 17 per cent of the sporocytes, and the same percentage of tetrads contained micronuclei. Bridge-fragment configurations, indicating chiasma formation in inverted segments, were found in 7 per cent of the sporocytes at second anaphase, although none was seen in the first division. As might be expected, 20 per cent of the pollen grains in this plant were abortive, while 22 per cent of the florets failed to set seed. This fertility, though slightly lower than that of other plants of the parent species, is nevertheless much higher than that of any of the hybrids.

Hybrids.—Meiosis in the hybrid *E. glaucus* × *S. jubatum* no. 3360 was much more regular than in any of the other hybrids studied. At first metaphase 81 per cent of the sporocytes had 14 bivalents, while the remainder had 13 bivalents and 2 univalents (table 2 and fig. 7). Chiasma frequency was highly variable, but lower than in the parents, the mean number of chiasmata per nucleus being 29. Because of this lower chiasma frequency, direct comparisons between the distribution of chiasmata in the hybrid and its parents could not be made, but the general appearance of the bivalents suggests an intermediate type of distribution. Except for the fact that the hybrid had fewer bridge-fragment configurations (2.9 per cent) than did *S. jubatum* (3.8 per cent), the percentage of irregularities was consistently higher than in the parents. Laggards were found in 10.4 per cent of the first anaphases and in 21.7 per cent of the second anaphases (table 4); while 17.6 per cent of the tetrads had micronuclei. These figures, however, are considerably lower than those for the other hybrids.

All of the other hybrids have less chromosome pairing at first metaphase (table 2) and more irregularities at later stages. In all of them, moreover, associations of three, four, five, and six chromosomes occur with a greater or lesser degree of frequency. In the hybrids between *Agropyron Parishii* and *Sitanion jubatum* (fig. 8), between *Agropyron* sp. "San Benito" and *Elymus glaucus* no. 549–2, and between *A.* "San Benito" and *Sitanion hystrix* (fig. 9), these consist only of chains or rings of three and four. These are present in 74 per cent of the sporocytes of *A. Parishii* × *S. jubatum,* but in only 7 and 14 per cent respectively of the other two hybrids. In the remaining hybrids, all of them involving *A. pauciflorum* as one parent, associations of five and six chromosomes were found in some of the sporocytes. These were least common in *A. pauciflorum* × *S. jubatum* no. 3388 (fig. 10), a fact probably due largely to the low amount of pairing in this hybrid, as indicated by the low chiasma frequency and the high proportion of univalents. In *A.* "San Benito" × *A. pauciflorum,* no. 548–3 (fig. 11), two different chains or rings were found in only one sporocyte. In the two hybrids between *A. pauciflorum* and *E. glaucus,* however,

sporocytes containing two such associations were relatively frequent. In both of them the maximum degree of chromosome association was found or was indicated to be a ring of six, a ring of four, and nine bivalents, except that in a single cell of the natural hybrid, CI no. 4330, two rings of six and eight bivalents were found (fig. 12, 13).

As shown in table 4, the hybrids which form higher associations at metaphase all have a high percentage of sporocytes with lagging chromosomes at the later meiotic stages, and in most of them the majority of the tetrads have extra nuclei. Bridge-fragment configurations, however, are relatively uncommon except in *A. pauciflorum* × *E. glaucus* no. 4330, and in *A.* "San Benito" × *A. pauciflorum* no. 548–3. As mentioned above, all of the hybrids, both natural and artificial, are nearly or quite devoid of pollen grains which stain normally, and all have to date proved completely sterile as to seed set, except when the chromosome number was doubled with the aid of colchicine.

Discussion.—The question arises as to whether the pairing which is observed in the hybrids is actually pairing between the complements of the two parents or whether it is partially or completely autosyndesis, i.e., pairing within each of the parental complements. The terms "autosyndesis" and "allosyndesis" refer here to pairing between chromosomes derived from the same or different parental gametes respectively. All of the species are tetraploids, with 2 genomes of 7 chromosomes each making up the gametic complement of 14 chromosomes. Since all the chromosomes are extremely similar in their morphology, no direct distinction is possible; therefore, only indirect evidence may be used in determining the nature of the pairing. Quadrivalents have not been observed in the parents, although a great many cells were examined in every case and although the chromosomes are long and the chiasma frequency high. The possibility cannot be overlooked, nevertheless, that the two genomes in a particular species might have some homology which, due to differential affinity between the chromosomes (Darlington, 1937), is not expressed in quadrivalent formation. Also the formation of bivalents in the parents might be genotypically controlled as suggested by Müntzing and Prakken (1940) in *Phleum*. If any such homology exists between the two genomes of *Elymus glaucus* or between those of *Sitanion jubatum,* however, it certainly is not expressed as autosyndesis in the hybrid between these two species. If all of the 14 pairs which are regularly formed are explained by autosyndesis, then there must be so much homology between the genomes of each parent that it would certainly result in quadrivalent formation at metaphase in the parents. It is not at all likely, then, that autosyndesis occurs in *Elymus glaucus* or *Sitanion jubatum* when these complements are combined with others in hybrids. *Sitanion hystrix* is so closely related to *S. jubatum* that a similar conclusion may almost certainly be made for its genome. There is good reason to believe, therefore, that in all of the hybrids at least the bivalents result mainly from allosyndesis.

That this is true at least in part for the higher associations is indicated by the following facts. First, they are nearly always simple rings or chains, characteristic of configurations resulting from structural hybridity. Second, in all of the hybrids involving *A. pauciflorum* associations of five or six chromosomes are found. Such high configurations in a tetraploid would have to be due at least in part to structural hybridity. Third, higher associations are most common in the hybrids involving *A. pauciflorum,* and these have a relatively low mean chiasma frequency and a high amount of chromosomal irregularity, indicating a relatively distant relationship between the parents. Finally the relationship between chiasma frequency in bivalents and that in higher associations is different from that found by other workers in autopolyploids or allopolyploids with a high proportion of duplicated segments. In tetraploids of *Tulipa,* Upcott (1939) found that in all but two species the frequency of half chiasmata per chromosome (or of chiasmata per two chromosomes) was higher in quadrivalents than in bivalents. The multivalents, therefore, were a selected sample of configurations with a high chiasma frequency. In the two exceptional cases where this was not true, the mean chiasma frequency per nucleus was lower than in the other species studied. In *Phleum pratense,* Myers (1944) found that chiasma frequency was also higher in quadrivalents than in bivalents. In the present material, however, exactly the reverse is the case. In all of the hybrids except *A. pauciflorum* × *S. jubatum,* chiasma frequency is definitely higher in bivalents than in the higher associations (table 2). Furthermore, this exceptional hybrid has the lowest chiasma frequency of all, and in the series as a whole there is a general correlation between high total chiasma frequency and a relatively high frequency in bivalents as compared to higher associations. This is exactly the reverse of the correlation found by Upcott in *Tulipa*. On the other hand, the frequency of half chiasmata per chromosome is lowest in associations of three, intermediate in those of four or five, and highest in rings and chains of six, as compared to other rings or chains. This situation can be explained if we assume that only certain chromosomes are capable of forming higher associations, and that these are a selected sample in which, because of large differences in segmental arrangement, the degree of pairing and chiasma frequency is lower than in the chromosomes which can form only bivalents, i.e., those in which the pairing mates derived from different parents are essentially similar in segmental arrangement. Within this group of segmentally rearranged chromosomes, chiasma frequency may vary at random, and the size of the ring or chain formed depends on these random variations, with higher chiasma frequencies producing larger associations. From this

we may conclude, therefore, not only that the higher associations are due chiefly or entirely to segmental interchange rather than to reduplication of chromosomes or genomes, but also that the maximum size and number of these associations found in a particular hybrid represents the degree of difference between its parents in the arrangement of large chromosomal segments.

Sterility.—As was pointed out elsewhere (Stebbins, 1945), the sterility of F_1 hybrids may be evident in the somatic tissue (diplontic sterility of Müntzing, 1930), or in the gametophytes (haplontic sterility of Müntzing, 1930). In the present material only haplontic, or gametophytic, sterility exists, since the sporophytic tissues, both vegetative and reproductive, are normal in every respect. This haplontic sterility may be either genic, and due to the genetic constitution of the plant (Dobzhansky, 1941, p. 293), or chromosomal, and due to structural dissimilarities between the parental chromosomes. If these dissimilarities involve large segments, pairing at meiosis will give rise to rings or chains and bridge-fragment configurations, but if the segments involved are small or minute and not very numerous, normal bivalent formation will be permitted. Nevertheless, the pairing of these dissimilar chromosomes will produce gametophytes and gametes containing deficiencies or duplications of chromosomal material, resulting in an unbalanced, disharmonious physiological system. Chromosomal sterility combined with apparently normal meiotic behavior will be found whenever the number of small structural differences between the chromosomes is large enough to cause this physiological upset of the gametophytes and gametes, but not so large that the number and size of the pairing blocks are reduced beyond the point where chiasma formation is possible. How many small structural differences can exist before this point is reached? This number undoubtedly varies greatly with the species in question. It depends chiefly on the length of the chromosomes and the chiasma frequency. The best available data are from *Zea mays*. Clarke and Anderson (1935) found normal bivalent pairing, but 50 per cent sterility, in a plant heterozygous for an interchange between the satellited and the third largest or $a\text{-}ts_4$ chromosome. The length of this translocated segment was about 8 per cent that of the entire $a\text{-}ts_4$ chromosome. Stadler (1933) found that a terminal deficiency involving 16 per cent of the total length of chromosome X could not be carried through the pollen, and gave megagametophytes of weakened vitality. McClintock (1938) found that an interstitial deficiency (Def-1) involving about 7 per cent of the pachytene length of chromosome V was not transmitted through the pollen, and later (1944) reported that deficiencies of from one to six terminal chromosomes, or about 3 to 12 per cent of the total length of chromosome 9 gave inviable pollen, while some even smaller deficiences gave pollen of weakened viability. We can probably assume, therefore, that the range of size of the chromosomal segments involved in cryptic structural hybridity, either through translocations or inversions, is from 1 to 10 per cent of the total length of the chromosome, and that the mean size of such segments is not over 6 per cent of the total length. In that case, if the mean chiasma frequency of the normal bivalent is two or higher, at least six of these small structural changes could be tolerated without preventing pairing and chiasma formation, since this would leave 64 per cent of the pairing length identical in the chromosomes affected. In diploid grass species with $n = 7$ and with chromosome size and chiasma frequency comparable to that found in the Hordeae, the chromosomes of two species could, on the basis of the evidence given above, differ by as many as 30 small interchanged or translocated segments and still produce an F_1 hybrid with good pairing but reduced chiasma frequency. On the other hand, this same evidence supports the postulate of Sax (1933), that a total of four or five small differences between the parental chromosomes would cause complete sterility. Hence in diploid hybrids of the tribe Hordeae, with seven potential bivalents, one might expect to find cryptic structural hybridity if chromosomal differences between the parental species involved between 5 and 30 small segments. In tetraploid hybrids like those described in this paper, with twice as many chromosomes, twice as many such differences could be present before pairing would be materially affected, but on the other hand the presence of duplicated chromosomal material would reduce the amount of sterility caused by a small number of these differences.

In such hybrids, the decision as to whether the sterility is genic or chromosomal must be made on indirect evidence, unless pachytene nuclei can be analyzed. Dobzhansky (1941) pointed out that in these critical cases the best method of distinguishing between these two types of sterility is furnished by doubling the chromosomal complement of the hybrid. Since in the polyploid produced by somatic doubling, pairing will be chiefly between exactly similar chromosomes, the allopolyploid will have greatly increased fertility if the sterility of the diploid F_1 was chromosomal. On the other hand, the balance between genic factors is not changed by somatic doubling, and therefore genic sterility will persist, although it might be somewhat changed as a result of different segregation ratios.

Among the present hybrids, all of those involving *A. pauciflorum* contain visible structural hybridity, and in addition have a relatively high frequency of univalents, both those caused by the failure to pair of potential bivalents, and those associated with chains of three or five. Spikes from colchicine-treated plants produced about 50 per cent of viable seeds, although as described above, no normal pollen grains or seeds have been found on undoubled plants, in spite of the thousands examined. In these hybrids, therefore, the sterility is partly or entirely chromosomal, and includes both visible and cryptic structural hybridity.

The hybrid *Elymus glaucus* × *Sitanion jubatum* has no visible structural hybridity and only slight reduction in the amount of metaphase pairing at meiosis. Nevertheless, the behavior of allopolyploid material obtained through somatic doubling by colchicine treatment provides strong indirect evidence that the sterility in this hybrid is chromosomal. Spikes from doubled sectors of treated plants yielded up to 80 per cent of viable seed, and the allopolyploid progeny grown from these seeds varied in fertility from 10 to 70 per cent. Only preliminary studies have been made of meiosis in these allopolyploids, but they indicate the following. Multivalents occur regularly, indicating a large degree of homology between the chromosomes of *Elymus* and *Sitanion* and supporting the evidence previously obtained on this point from the diploid hybrid. The number of multivalents, mostly 5–6 per nucleus, is slightly less than might be expected on the basis of data from artificial and putative natural autotetraploids of grass species with large chromosomes, in which 50 to 60 per cent of the chromosomes are usually associated as multivalents (Myers and Hill, 1940; Myers, 1945). This indicates that the chromosomes of *E. glaucus* and *S. jubatum* are different enough to bring about preferential pairing between the two sets of chromosomes derived from the same parental species. Hence all of the evidence points toward the fact that the F_1 hybrid between *E. glaucus* and *S. jubatum* is a typical case of chromosomal sterility due to cryptic structural hybridity.

The hybrids *A. Parishii* × *S. jubatum*, *A.* "San Benito" × *E. glaucus*, and *A.* "San Benito" × *S. hystrix* are intermediate between the two extreme types mentioned above. In *A. Parishii* × *S. jubatum* spikes from doubled sectors of colchicine-treated plants had about 50 per cent of seed fertility, but the progeny from these seeds have not yet flowered. On the basis of this and the sum total of evidence given above, the most likely conclusion is that in these three hybrids the sterility also is mainly chromosomal, with cryptic structural hybridity the most important factor.

Cytological relationships between the parental species.—As already pointed out, the somatic chromosome complements of the parental species are indistinguishable from each other. The cytological differences between them are therefore confined (1) to differences in frequency and position of chiasmata at meiotic metaphase in the species themselves, and (2) to differences in the arrangement of chromosomal segments, as evidenced by the type of pairing found in the hybrids.

In respect to the chiasma differences, the species investigated can, as was described above, be arranged in a series, starting with *A. Parishii*, which has the lowest chiasma frequency and the least variation in the frequency of chiasmata per nucleus, per chromosome and per chromosome arm, and ending with *S. jubatum*, which has the highest frequency and the greatest variability in distribution of chiasmata. A noteworthy fact is that these two species are at opposite extremes in respect to two other characteristics, namely, size of anthers and amount of cross vs. self pollination. In *A. Parishii*, the anthers are nearly 6 mm long, and they are always well exserted before they shed their pollen, while in *S. jubatum* they are 1.8 mm long and often fail to become exserted, depositing their pollen on the stigma of the same floret. Consequently, *A. Parishii* is largely cross pollinated and heterozygous, as shown by the variability within seedling progenies from a single parent, while *S. jubatum* is chiefly self-pollinated and homozygous, as shown by the uniformity of its seedling progenies. *Elymus glaucus* is intermediate in all of these characteristics, while *A. pauciflorum* resembles *E. glaucus* in chiasma frequency and distribution, and in the amount of exsertion of the anthers, although in anther size it is the same as *S. jubatum*.

This correlation hardly seems accidental, and examination of other species of the genera for these characteristics seems desirable. Two hypotheses occur to the writers which might explain the situation. In the first place, the initial chiasma frequency and distribution may be the same in all of the species concerned, and the differences at metaphase may be due to a greater amount of movement of chiasmata in *A. Parishii*. In *Bromus carinatus*, which may produce some florets with small anthers and others with large ones on the same panicle, Harlan (1945a) found that meiosis proceeds more rapidly in the small anthers than in the large ones. If this is true in the species of the Hordeae under discussion, then the slower meiosis in *A. Parishii* could permit a greater amount of chiasma movement than in the other species. Against this hypothesis are the following facts. In the first place, complete terminalisation of the chiasmata in a bivalent is uncommon even in *A. Parishii*. Furthermore, as was described above, observation of sporocytes at early diakinesis in *A. Parishii* indicates that the movement of chiasmata between that stage and metaphase is slight, and that consequently the differences between the species concern the actual incidence of chiasmata, and therefore of crossing over (Darlington, 1937). This leads to the second hypothesis, namely, that the correlation between chiasma frequency and distribution, anther size, and amount of cross pollination is due not to a direct developmental relationship between these factors, but to a selective advantage of the combination of characters found. In *A. Parishii*, individual variability and heterozygosity are secured in every generation by means of cross pollination. Consequently the additional amount of gene recombination which would be caused by a large amount of crossing over does not have a high selective value. In the other species, however, predominant self pollination has led to a variation pattern similar to that in *Bromus carinatus* (Harlan, 1945b), consisting of a large number of homozygous or nearly homozygous races, with heterozygosity obtained periodically through occasional crossing between races. The ability of

such species to adapt themselves to new conditions would depend largely on the ability of the heterozygous interracial hybrids to produce many new gene recombinations, some of which could have selective value, and thus form the beginning of a new race. Under these conditions, any factor increasing the amount of gene recombination would have an enhanced selective value, and this would hold for

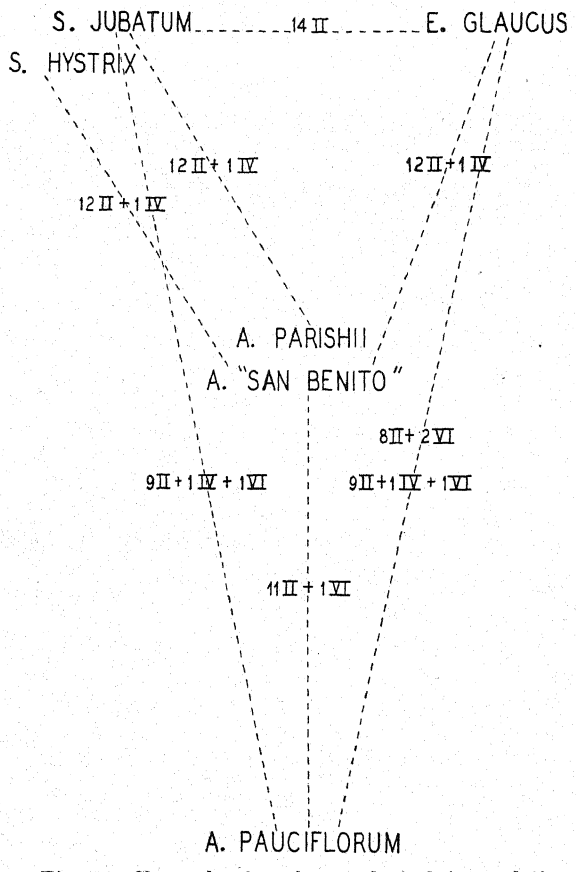


Fig. 14. Chart showing the cytological interrelationships of the species studied, based on the maximum number and size of the interchange rings or chains found at meiosis.

reduction of the amount of linkage by a higher chiasma frequency and frequency of crossing over. We may thus postulate that as a result of the adoption by *Sitanion* of predominant self pollination, mutations in the direction of increased chiasma frequency have spread through the species because of their higher selective value. If this hypothesis is correct, a similar correlation between self-pollination and high chiasma frequency should exist throughout the tribe Hordeae, and perhaps the Gramineae as a whole.

If we accept this latter interpretation of the origin of the differences in chiasma frequency and distribution between the species, then we should not expect any correlation between these differences and the degree of difference in chromosome homology as shown by the amount of irregularity at meiosis. This is what we find. *Elymus glaucus* and *Sitanion jubatum,* which differ conspicuously from each other in chiasma frequency and distribution, form a hybrid with nearly regular meiosis, while meiosis in the hybrid between *E. glaucus* and *A. pauciflorum,* which have essentially the same chiasma frequency and distribution, is much more irregular.

The other cytological criteria which might be used to indicate degree of relationship are first, the amount of difference between the species in regard to inverted segments, as expressed by the frequency of bridge-fragment configurations in the hybrid; second, the amount of rearrangement of large chromosomal segments, as expressed by the size and frequency of rings or chains in meiosis of the hybrids; and third, the degree of homology between the chromosomes, as shown by chiasma frequency and the occurrence of unpaired univalents. In respect to bridge-fragment configurations most of the hybrids have frequencies lower than that found in one of the parental species, *Sitanion jubatum* (table 4). Relatively high frequencies are found only in two hybrids involving *A. pauciflorum,* namely, the natural one between this species and *E. glaucus* (CI 4330) and *A. pauciflorum* × *A.* "San Benito." In *A. pauciflorum* × *S. jubatum* the frequency of bridge-fragments is higher than in *S. jubatum* itself, and in view of the low chiasma frequency in this hybrid, this frequency undoubtedly indicates more heterozygosity for inversions. The evidence from inversion heterozygosity, therefore, indicates that *A. pauciflorum* is relatively remotely related to the other species.

The evidence from ring and chain configurations is summarized in the diagram, figure 14. This gives the maximum size and number of such configurations found or expected in any one cell of each hybrid, and thereby the degree of interchange heterozygosity for segments large enough to produce multivalents. The reasons for assuming that these associations are the result of interchange heterozygosity have been given above. Figure 14 shows that in this respect *A. pauciflorum* is relatively remotely related to the other species, that *Elymus* and *Sitanion* are close to each other, and that *A. Parishii* and *A.* "San Benito" are intermediate, but nearer to *Elymus-Sitanion* than to *A. pauciflorum.*

The distribution of chiasma frequencies in the hybrids points in the same general direction. When the three hybrids involving *E. glaucus* are compared, that with *S. jubatum* is seen to have the highest mean chiasma frequency, 2.07 chiasmata per two chromosomes, while those with *A.* "San Benito" and *A. pauciflorum* are lower, 1.88 and 1.86. Comparing similarly the hybrids involving *S. jubatum* or *S. hystrix,* the highest frequency is again in that with *E. glaucus,* the lowest (1.33) in that with *A. pauciflorum,* and those with *A. Parishii* and *A.* "San Benito" (1.61 and 1.62) are intermediate. Similarly, in the case of *A.* "San Benito," the hybrid with *E. glaucus* has the highest, that with *S. hystrix* an intermediate, and that with *A. pauci-*

florum the lowest chiasma frequency. Obviously, two factors are of primary importance in controlling the chiasma frequency of these hybrids; first the genotypically controlled chiasma frequency of the parents, and second the amount of pachytene pairing in the hybrids, as determined by the degree of homology of the parental chromosomes. If the first factor has the greatest effect, one would expect the high frequency found in *Sitanion* to be reflected in a higher range of chiasma frequencies in its hybrids. Actually, with the exception of *E. glaucus* × *S. jubatum,* the reverse is the case, since the various hybrids between *Agropyron* spp. and *Sitanion* have in every instance a lower chiasma frequency than hybrids of the same *Agropyron* species and *E. glaucus.* We must conclude, therefore, that the differences in chiasma frequency reflect chiefly differences in amount of pairing, and therefore in chromosome homology. These differences are most likely due to rearrangements of small chromosomal segments, or cryptic structural hybridity. The evidence suggests, therefore, that the amount of structural differentiation between the chromosomes of the parental species is about the same for all types of chromosomal changes, and that figure 14 is a rough approximation of the degree of cytological relationship between them.

It is noteworthy, however, that the variation in frequency of univalents between the various hybrids does not fall into this pattern. The discrepancy involves those hybrids with either *Agropyron* "San Benito" or *A. Parishii* as one parent. Thus *A.* "San Benito" × *E. glaucus* and × *S. hystrix* both have much higher frequencies of univalents than might be expected on the basis of their relatively high chiasma frequencies, while in *A.* "San Benito" × *A. pauciflorum* and *A. Parishii* × *Sitanion jubatum,* these values are unexpectedly low. No explanation for this anomalous situation is at hand, but it seems likely that genic complexes are present in *A.* "San Benito" and *A. Parishii* which affect chromosome behavior in their hybrids, independently of the degree of chromosome homology. As shown in table 4, the amount of lagging of chromosomes at later stages of meiosis is in general correlated with the frequency of metaphase univalents.

As is evident from the different generic names which have been used, the taxonomic position usually assigned to the species under discussion corresponds only in part to their relationships as determined by cytogenetic evidence. From the cytogenetic point of view, there is no reason at all for maintaining the genus *Sitanion* as distinct from *Elymus,* since the ease of crossing and cytology of the hybrid indicate that *E. glaucus* and *S. jubatum* are closely related species. In fact, preliminary data indicate that these two species are more closely related to each other than *E. glaucus* is to *E. canadensis,* two species which are usually placed very near each other in systematic treatments. In regard to *Agropyron* and *Elymus,* the situation is much more complex, since both of these genera are large and contain many diverse species groups. The present evidence, however, would indicate that the forms studied here should all be placed in the same genus, and that the only character used by systematists to separate *Agropyron* and *Elymus,* namely, whether the spikelets are single or more numerous at the nodes of the rachis, is actually of no greater importance as an indicator of relationship than are several other characters. *A. Parishii* and *A.* "San Benito" are placed in *Agropyron* because their spikelets are normally borne singly, although in both species vigorous spikes on cultivated plants may bear two spikelets at the central nodes of the spike. There are, however, many resemblances between these two species and *E. glaucus* in general habit, as well as in the morphology of the glumes, lemmas, paleae, and lodicules. In fact, *E. glaucus* is in these respects more similar to *A. pauciflorum* than it is to such other *Elymus* species as *E. condensatus* Presl., *E. triticoides,* Buckl., and *E. arenarius* L., the latter of which is the type species of *Elymus.*

When we compare the meiotic behavior in the present hybrids with that in interspecific hybrids of *Aegilops* (Kihara, 1940; Sears, 1941), the genus of the tribe Hordeae most thoroughly studied in this respect, we find that the present hybrids are much less irregular and show a greater degree of affinity between the parental chromosomes than all but a few of the *Aegilops* hybrids. *Elymus glaucus* × *Sitanion jubatum* resembles *Ae. speltoides* × *sharonensis,* an intrasectional hybrid between two closely related species, and one of the most regular of all of the *Aegilops* hybrids. *Agropyron pauciflorum* × *Sitanion jubatum,* the most irregular of the present hybrids, is not quite as irregular as the most irregular intrasectional hybrid in *Aegilops, Ae. comosa* × *uniaristata,* and does not approach the irregularity of most of the intersectional hybrids. When compared with the species of *Aegilops,* therefore, *Elymus glaucus, Sitanion jubatum, S. hystrix, Agropyron Parishii,* and *A. pauciflorum* behave cytogenetically as if they belonged to the same section or subgenus rather than to different genera. The same is true when they are compared with *Bromus,* the genus of Gramineae most nearly related to the Hordeae. Knowles (1944) found much more irregular meiosis in the intrasectional hybrid *B. mollis* × *arenarius* than exists in any of the present hybrids, and such intersectional hybrids as he obtained were even more irregular. The senior author (unpubl.) has similarly found great meiotic irregularity in all of the intersectional hybrids of *Bromus* which he has obtained, and conditions similar to that in *E. glaucus* × *S. jubatum* are found only in hybrids between such closely related species as *B. laevipes* Shear and *B. vulgaris* Shear or *B. grandis* Shear. The cytogenetic evidence, therefore, would suggest that a drastic revision of the present systematic treatment of the tribe Hordeae

is necessary, but the nature of this revision will not be clear until many more hybrids have been studied.

SUMMARY

The following natural F_1 hybrids, occurring with their parents, have been found in California: *Elymus glaucus* × *Sitanion jubatum*, *Agropyron pauciflorum* × *S. jubatum*, *A. Parishii* × *S. jubatum*, and *A. pauciflorum* × *E. glaucus*. The last named hybrid has been reproduced artificially, and in addition artificial hybrids have been obtained between an unidentified species of *Agropyron* (*A.* "San Benito") related to *A. Parishii* and *A. pauciflorum*, and *Elymus glaucus*, *Sitanion hystrix*, and *A. pauciflorum*. All of these hybrids, both natural and artificial, are intermediate between their parents in external morphology, including those taxonomic characters used to separate the genera to which they have been assigned. All of them are completely sterile in the original condition, but from some of them fertile offspring have been obtained by doubling the chromosome number with the aid of colchicine. *Elymus glaucus* × *Sitanion jubatum* corresponds to the "species" recognized in the manuals as *Sitanion Hanseni* (Scribn.) J. G. Smith, while *Agropyron pauciflorum* × *S. jubatum* resembles *A. Saundersii* (Vasey) Hitchc.

All of the species and hybrids are tetraploids with 2n = 28 chromosomes, and the external morphology of the chromosomes is essentially the same in all of them. In all of the species 14 bivalents are regularly formed at meiosis, and the divisions are essentially regular. The species fall into three groups in respect to the frequency and distribution of chiasmata. *Agropyron Parishii* has the lowest frequency and the least variability, with usually 2 chiasmata per bivalent, one in each arm. In *Elymus glaucus* and *A. pauciflorum* chiasma frequency is slightly higher, and a larger proportion of chromosome arms have no, as well as two or three, chiasmata. *Sitanion jubatum* has a still higher chiasma frequency and more variable distribution. These differences are believed on indirect evidence to represent differences in the incidence of chiasmata rather than in terminalisation. They are correlated with the degree of cross vs. self fertilization which ordinarily occurs in the species concerned.

Meiosis in *E. glaucus* × *S. jubatum* is nearly regular in spite of its sterility. Indirect evidence, however, particularly the situation in the allopolyploid derivative, indicates that its parents differ by small or minute structural rearrangements, and that the sterility is due to cryptic structural hybridity.

In all of the other hybrids, meiosis is more irregular, with a lower chiasma frequency at first metaphase, and a high proportion of irregularities at later stages. In addition, ring or chain configurations are found in all of them. These may involve five or six chromosomes in all of the hybrids involving *A. pauciflorum* as one parent, but associations of only three or four were found in the other hybrids. The behavior in the parents indicates that the bivalents in all of the hybrids result from allosyndesis. The form of the higher associations as well as their relatively low chiasma frequency indicates that they also result from allosyndesis, and are the product of interchanged rather than reduplicated chromosome segments. In these hybrids, therefore, sterility results from visible as well as cryptic structural hybridity.

All of the cytological evidence indicates that *E. glaucus* and *S. jubatum* are as closely related to each other as species belonging to the same section or subgenus. Of the other species considered, *A. pauciflorum* is the most distantly related to these two, while *A. Parishii* and *A.* "San Benito" are intermediate in their relationships.

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EFFECT OF HYDROGEN-ION CONCENTRATION ON THE TOXICITY TO COLLETOTRICHUM CIRCINANS (BERK.) VOGL. OF SOME CARBOXYLIC ACIDS, PHENOLS, AND CRUCIFER EXTRACTS [1]

Frederick G. Smith, J. C. Walker, and W. J. Hooker

DURING A biochemical study of resistance to the clubroot of crucifers, a large number of toxicity tests were made on various extracts of host tissue. Toxicity was determined by the inhibition of germination of spores of *Colletotrichum circinans* (Berk.) Vogl. This fungus has been commonly used in this laboratory (Walker, Morell, and Foster, 1937; Pryor, Walker, and Stahmann, 1940; and Hooker, Walker, and Smith, 1943) as a test organism, since *Plasmodiophora brassicae* Wor., the incitant of clubroot, is an obligate parasite and difficult to use in toxicity testing (Hooker, Walker, and Link, 1945. In the course of these tests it was observed that the hydrogen-ion concentration of certain acidic fractions had a marked and characteristic effect on toxicity. It became necessary, therefore, to investigate this effect further in regard, first, to the technic of routine toxicity testing and, second, to the possibility that a better understanding of the effect might offer some clues to the type of toxic compounds involved in clubroot resistance.

The effect of hydrogen-ion concentration on the bactericidal or bacteristatic properties of certain types of compounds has been reported several times. Goshorn *et al.* (1938) and Bittenbender *et al.* (1940) showed that pH markedly influenced the bacteristatic effects of several common antiseptics including phenols and carboxylic acids on *Staphylococcus aureus* and *Bacterium coli*. More recently Rahn and Conn (1944) have made a more thorough study of the effect of hydrogen-ion concentration on the toxicity of benzoic, salicylic, and sulfurous acids on yeast and *B. coli*. They observed that it is generally only the weaker disinfectants that show changes in efficiency at different pH levels.

Less attention has been paid to the effect of hydrogen-ion concentration on fungicidal action although there have been several studies of the general fungicidal properties of phenols and carboxylic acids (Cook and Taubenhaus, 1912; Tetsumoto, 1933a, 1933b; Walker and Link, 1935; Rigler and Greathouse, 1940; Greathouse and Rigler, 1940). However, recently Hoffman, Schweitzer, and Dalby (1939, 1941) have pointed out that several antiseptics and related compounds showed marked differences in efficiency with changes in hydrogen-ion concentration. Phenols and carboxylic acids in particular gave characteristic curves relating pH and inhibitory concentrations. They measured toxicity by the minimum concentration of the compound in phosphate-citrate buffered agar necessary to inhibit visible growth of a mixed culture of common molds occurring on food stuffs. With phytopathogenic fungi germination methods have been more commonly used in the assay of fungicides and are capable of more precise control. There has, however, been little if any reference to the possible effect of hydrogen-ion concentration on toxicity measurements by this technic.

METHODS AND MATERIALS.—The following germination test was designed for the assay of minimum quantities of extracts with maximum economy of time and effort consistent with good precision. The technic of the assay is similar in many respects to that recently described by the Committee on Standardization of Fungicidal Tests of the American Phytopathological Society (1943). Aqueous solutions of test materials were prepared in 0.5- to 2.0-ml volumes. Ethyl-ether concentrates of the crucifer extracts were evaporated into water in test

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tubes by shaking in a 50°C. water bath with a jet of air on the ether surface, and traces of ether were removed from the solution by concentrating *in vacuo* to about three-quarters of the original volume. The solution was then restored to the original volume, after pH adjustment in appropriate cases, and suspended fatty material was largely removed by suction filtration through a layer of washed super-cel. Serial dilutions of 0.2- to 0.4-ml of the resulting solutions were made in test tubes with serological pipettes to give dose ratios of 1.5 or 2 and an equal volume of spore suspension was added.

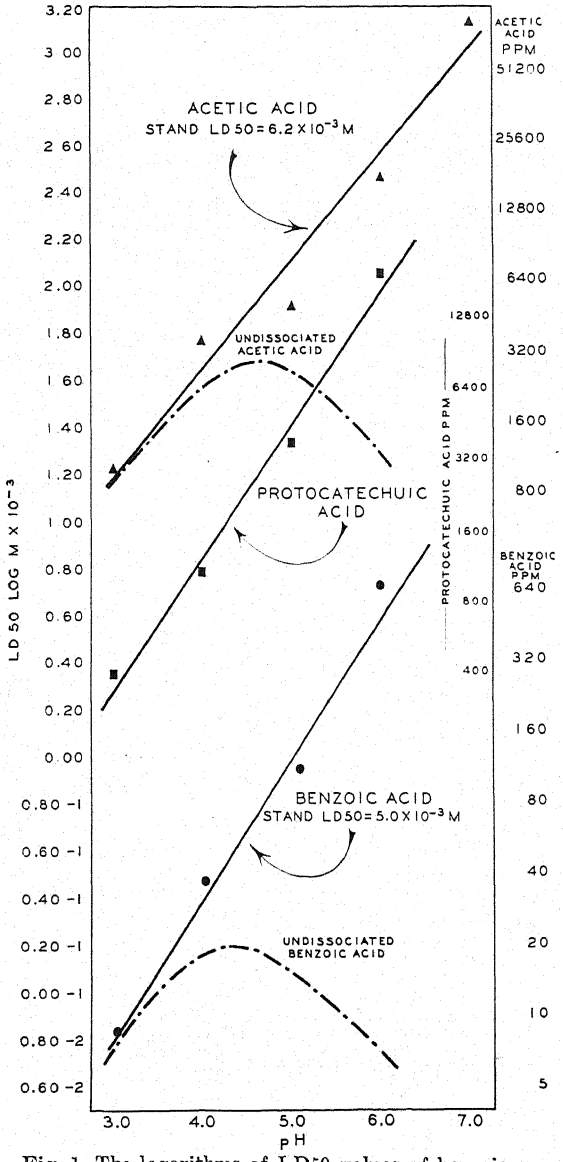


Fig. 1. The logarithms of LD50 values of benzoic, protocatechuic, and acetic acids as functions of pH. The dotted curves represent the calculated undissociated acid concentration corresponding to the linear estimate of total acid and acid anion concentration at various pH's.

Spore suspensions of *C. circinans* prepared from 10- to 14-day cultures grown on potato-dextrose agar were filtered and centrifuged through two changes of distilled water and adjusted in the appropriate buffer to 2000 spores per mm^3 using a Fuchs-Rosenthal cell. Four replicate drops on two slides were prepared in each series of concentrations and incubated 20 to 24 hours at 23°C. in a petri-dish moist chamber. The pH was determined on the remaining aliquot of each dilution with a Beckman pH meter.

The "observed per cent germinated" was determined by counting 25 spores in one microscopic field in each drop. "Corrected per cent inhibited from germinating" was calculated by subtracting from the control per cent germinated the observed per cent germinated, multiplying the difference by 100 and dividing the product by control per cent germinated (Abbott's correction for natural mortality). These values were plotted against toxicant concentration on logarithmic-probability coordinates and the LD50 values determined by visual fitting of the best straight line. The majority of the trials gave from three to five points of nearly linear distribution with relatively small and quite inconsistent variations in slope among toxicants and pH's, except for occasionally distinctly smaller slopes, at pH 7. Tests of error for replicate counts by the chi-square method (McCallan and Wilcoxon, 1932) showed, with few exceptions, that variation could be accounted for by sampling error. The spore sensitivity distribution was found on several trials to be essentially normal. As a check on variation in sensitivity of spore lots, a standardization series using protocatechuic acid as the toxicant was included in each day's run. When occasional large departures from the average response to protocatechuic acid were obtained, the data were rejected.

The crucifer extracts were ether-soluble, strong acid fractions of root tissue, preparation of which will be described elsewhere.

EFFECT OF HYDROGEN-ION CONCENTRATION ON TOXICITY.—*Hydrogen-ion concentration "alone" and "non-toxic" buffers.*—In examining the effect of hydrogen-ion concentration on the toxicity of pure compounds or extracts it is necessary to separate any possible direct effect of hydrogen ions themselves on germination. Fungi commonly germinate over wide ranges of hydrogen-ion concentration sometimes down to pH 2 or 2.5, though for any particular species the range may be narrower. It was important in the present work to know more accurately how the viability of the spore population as measured by the extent of germination would vary with pH. Investigation was limited to the range of pH 3 to 7 which covers the physiological range of host tissue. The problem of measuring the toxic effect of hydrogen ions "alone" rests on the selection of "non-toxic" buffers. Assuming that variation in concentration of sodium ions would have negligible effect, acid-salt mixtures of five acids of suitable dissociation constants were tested, namely, citric, acetic, lactic, oxalic, and phosphoric.

Of these, phosphoric acid provided the highest consistent germination over the range from pH 3 to 7 and was used in all the following work at a final concentration of 0.1M, although it was recognized that at the lower part of the range the buffering capacity was not great. The results of five typical sets of controls are shown in table 1. Analysis of variance showed no significant difference in germination over the range of pH 3 to 6 but germination was significantly reduced at pH 7. Experiments whose control germination dropped below these values were discarded. It should be pointed out that Abbott's correction is not altogether satisfactory for the natural mortalities encountered in this work, especially those at pH 7. Furthermore, no adequate test of the normality of sensitivity distribution was made at pH 7.

TABLE 1. *Per cent spore germination of C. circinans in phosphate buffer at various hydrogen-ion concentrations.*

	Replicate number					
pH	1	2	3	4	5	Average
3	85	85	95	88	80	86.6
4	91	89	89	87	85	88.2
5	84	87	92	92	74	85.8
6	86	80	94	89	63	82.4
7	57	43	44	47	38	45.8

Carboxylic acids.—The LD50 values of three carboxylic acids, benzoic, protocatechuic, and acetic, as functions of pH are given in figure 1. These are representative of three common naturally occurring types of carboxylic acids: a pure aromatic, a phenolic, and an aliphatic acid, respectively. All three showed a rapid decline in toxicity with pH, benzoic being about 30 times more toxic than protocatechuic which in turn was about five times more toxic than acetic at each pH. The relation between log LD50 and pH was, in fact, nearly linear in each case.

It was suggested by Clark (1899), Vermast (1921), and Eggerth (1926) and again by Rahn and Conn (1944) for bacteria and yeast and by Tetsumoto (1933a, 1933b) and Rigler and Greathouse (1940) for fungi that the undissociated acid molecule is the toxic entity and, therefore, that hydrogen-ion concentration affects toxicity by controlling the degree of dissociation of the acid. The validity of this explanation for the present data was tested by computing the concentration of undissociated acid molecules corresponding to the best straight line through the LD50 data for total acid anion concentration. If the undissociated acid were the only toxic molecular species and there were no variation in spore sensitivity distribution with pH, the curve of LD50 against pH for undissociated acid would be a straight line parallel to the pH axis. Figure 1 shows that this is not the case, for the undissociated acid curves are parabolic with maxima between pH 4 and 5. This, however, does not necessarily refute the hypothesis since it was not proven that there was no variation with pH in the sensitivity distribution of the spores. The evidence available is the fairly constant percentage germination in the buffer controls up to pH 7. It would be necessary to have more data on the distribution of spore sensitivity with pH of other fungicides whose chemical properties do not change with pH and the toxicity of which, therefore, should not. In previous work (Walker and Link, 1935; Rigler and Greathouse, 1940; Greathouse and Rigler, 1940; Hoffman, Schweitzer, and Dalby, 1939, 1941) the minimum concentration to inhibit growth was used as the measure of toxicity so it was the sensitivity of the most resistant individuals in the population, not of the mean, that determined the response.

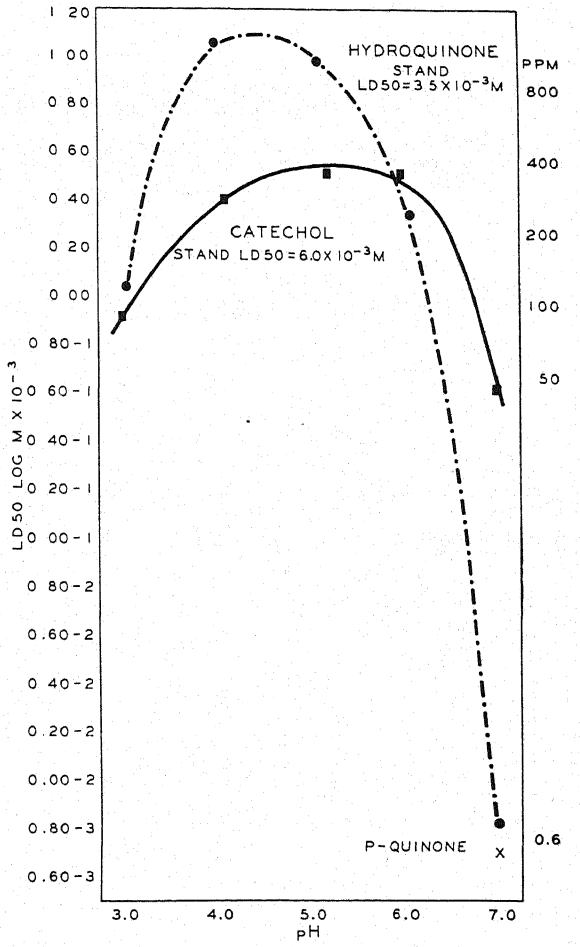


Fig. 2. The logarithms of LD50 values of catechol and hydroquinone as functions of pH from 3 to 7 and p-quinone at pH 7.

Phenols.—The LD50 values for hydroquinone and catechol as a function of pH are given in figure 2. The pH-toxicity relationship of these two phenolic compounds is in marked contrast to the straight line relations of the three carboxylic acids shown in figure 1. In both cases toxicity was considerably greater at pH 3 and 7 than at 4 and 5. Solutions of catechol and hydroquinone autoxidize

TABLE 2. *Rates of autoxidation of hydroquinone, catechol, and protocatechuic acid, 0.01 M, in 0.1 M phosphate buffers at 30°C. in Warburg apparatus*

Expt.		Cubic millimeters O_2 uptake								
		hydroquinone			catechol			protocatechuic acid		
no.	Minutes	pH 5	6	7	5	6	7	5	6	7
	100	0	0	2	0	0	9	0	0	0
1	240	0	0	15	1	0	16	0	0	4
	420	7	10	28	1	0	32	0	0	6
	35	0	0	1	0	0	2	0	0	0
	90	0	0	8	0	0	8	0	0	0
	160	0	0	17	0	0	17	0	4	5
2	260	7	8	26	6	4	28	3	11	12
	360	17	18	42	14	14	44	17	21	21
	500	26	26	65	26	26	68	29	35	35

at room temperatures especially at higher pH's (cf. table 2) to produce quinones which have been reported to be much more powerful germicides than their corresponding phenols (Morgan and Cooper, 1921; Thalhimer and Palmer, 1921; Cooper and Mason, 1927). This may explain the marked increase in toxicity at pH 7 where autoxidation occurs most rapidly. In the case of hydroquinone whose quinone is relatively stable, it was possible to show that at pH 7 hydroquinone and p-quinone did, in fact, have about the same toxicity (fig. 2) indicating that most of the hydroquinone had apparently been oxidized. The smaller effect with catechol may be due to the fact that its quinone is much less stable at pH 7 (Onslow, 1931; Willstätter and Pfannenstiel, 1904) and may have broken down quickly into less toxic compounds. It should be borne in mind that germination in the controls was consistently lower at pH 7 (table 1) than at lower pH's. This may indicate altered spore sensitivity distribution which would make toxicity determinations less reliable at this pH. It is interesting to note that protocatechuic acid which is also an orthodihydroxyphenol showed no evidence of catechol-like behavior in its pH-toxicity relations. This would follow from the smaller tendency toward autoxidation in the carboxyl-substituted phenolic ring as indicated in table 2.

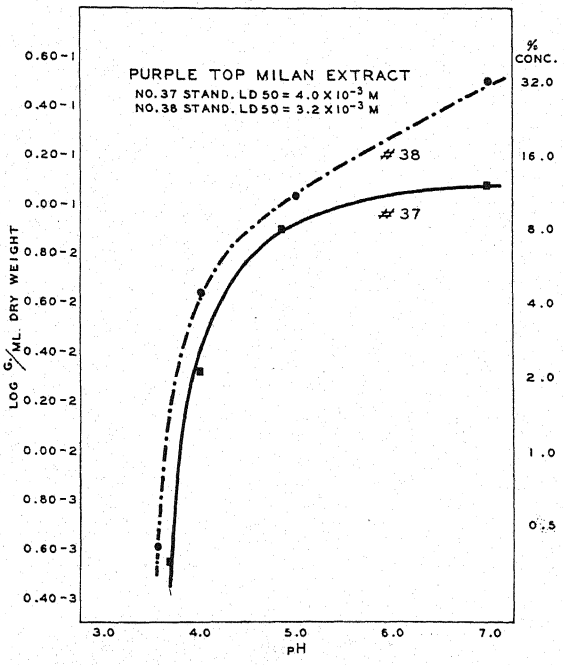


Fig. 3. The logarithms of LD50 values for a crucifer extract as a function of pH.

Crucifer extracts. — Preliminary observations showed that neutralization of the strong acid fractions to pH 6 caused a 16- to 32-fold drop in toxicity. The reaction of the unneutralized extracts varied from pH 3 to 5.7 and it was clear that the toxicity of compounds in the extracts varied markedly with hydrogen-ion concentration. Investigation of the influence of hydrogen-ion concentration on toxicity of numerous samples showed a characteristic relation of LD50 and pH, illustrated by duplicate curves for a turnip extract (Purple Top Milan) in figure 3. Comparison with pH-toxicity curves for typical phenols and carboxylic acids seemed to indicate that a large part of the toxicant action was that of carboxylic acids or compounds having similar behavior. The fact that most of the fractions showed a plateau in the curves above pH 6 also may have indicated the presence in smaller amounts of compounds whose toxicity did not depend so markedly on hydrogen-ion concentration. This conclusion was supported by other analytical data.

DISCUSSION.—The influence of hydrogen-ion concentration on the toxicity of the crucifer extracts indicated the necessity for careful control of pH in the routine assay of various fractions and varieties in disease resistance work. The characteristic pH-toxicity relations of the extracts and of the typical phenols and carboxylic acids to fungi confirmed earlier reports (Hoffman, Schweitzer, and Dalby, 1939, 1941) and offers a possible clue to the identity of the natural fungicidal compounds in crucifers.

This pH-toxicity relation also suggests that other classes of fungicides, naturally occurring and com-

mercial, especially those of acid character, may show significant variation in toxicity with hydrogen-ion concentration. Considering that a pH difference of one unit may cause a 3-fold change in toxicity with carboxylic acids or a much greater change with a phenol of the hydroquinone type near neutrality, it seems likely that this phenomenon may have some practical importance for commercial fungicides. It would be especially true in the laboratory testing of compounds sufficiently soluble and acidic to alter the pH of poorly buffered media.

Cell sap acidity and pH as factors in natural disease resistance have been investigated for a number of diseases (Cook and Taubenhaus, 1912; Walker, 1923; Sherwood, 1923; Scott, 1924; Hurd-Karrer, 1925; Newton, Lehmann, and Clarke, 1929) without very conspicuous success. Even in those cases where a clear correlation seemed to exist the differences in pH between resistant and susceptible tissue were often small enough to make it difficult to understand how they could influence resistance to pathogens capable of growing *in vitro* over much wider pH ranges. In the foregoing effect of pH on toxicity lies a possible explanation. Small differences in hydrogen-ion concentration of resistant and susceptible tissue may cause relatively large differences in the fungicidal effectiveness of certain cellular constituents. The observed behavior of hydroquinone and catechol, representatives of the widely occurring ortho- and para-dihydroxyphenolic compounds, suggested that hydrogen-ion differences also may influence toxicity by affecting the rate or extent of conversion to the more toxic quinones on invasion by pathogens or by other injury. Higher pH's would not only facilitate autoxidation but would tend to accelerate the enzymatic oxidation of phenols by polyphenol oxidase whose optimum pH is about 7 (Raper, 1932). It is suggested, therefore, that relatively small differences in hydrogen-ion concentration may have greater significance in disease resistance than has been generally believed.

SUMMARY

It was observed that the toxicity of ether-soluble, strong acid fractions of crucifer extracts was correlated with pH. A study of the variation in toxicity with pH of extracts and several typical phenols and carboxylic acids was made by determining the per cent spore germination. Plotting logarithms of LD50 values against pH gave characteristic curves for each group of toxicants.

The carboxylic acids showed nearly parallel linear curves with toxicity decreasing in order: benzoic, protocatechuic, and acetic acids. Calculated pH-LD50 curves for the undissociated acid fraction did not altogether support the hypothesis that this molecular species is responsible for the toxicity of carboxylic acids at various hydrogen-ion concentrations.

Hydroquinone and catechol showed distinctly different curves than the acids. The marked increase in toxicity at pH 7 especially with the former and comparison with the toxicity of the corresponding p-quinone suggested that autoxidation to quinone had taken place.

The curves for crucifer extracts indicated that carboxylic acids or similar toxicants were largely responsible for toxicity.

These results suggest two possible mechanisms by which relatively small differences in pH may produce changes in fungicidal activity of importance in natural disease resistance or in the application of commercial fungicides.

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AN ELECTRON MICROSCOPE STUDY OF TOBACCO MOSAIC VIRUS AT DIFFERENT STAGES OF INFECTION [1]

T. E. Rawlins, Catherine Roberts, and Nedra M. Utech[2]

At the present time little information is available regarding the mode of multiplication of viruses. Stanley (1937) found that a given weight of purified tobacco mosaic virus from plants infected for a week produces fewer local lesions than the same weight of purified virus from plants that had been infected for four weeks. Spencer (1942) studied the infectivity of purified tobacco mosaic virus from leaves that had been infected for five days and those infected for 20 days. A given weight of virus from leaves infected 20 days was about 37 per cent more active in the production of local lesions than the same weight of virus from leaves infected for five days. A single preliminary determination of sedimentation rates of the two lots was interpreted to indicate that the 20-day virus has particles of approximately uniform size, whereas 5-day virus has some particles of the same size and some particles nearly double this length. Unfortunately Spencer was unable to continue this work and the evidence was therefore left in an inconclusive state.

If it were proved, as Spencer indicated, that a leaf infected for five days contains some particles around twice the typical length and that a similar leaf infected for 20 days then contains only particles having the typical length of around 300 mμ, it would be reasonable to conclude that between the fifth and the twentieth days of infection the double length particles have probably undergone transverse fission to produce the 300 mμ particles. This would account for the virus multiplication that is known to occur during this period, and for the cessation of virus multiplication in 20-day infected leaves, noted by Spencer.

Accordingly we have attempted to clarify this situation by using the electron microscope to study tobacco mosaic virus from leaves in different stages of infection.

Methods.—The methods were similar to those used by Spencer. One to three lateral leaves of Turkish tobacco plants were inoculated with tobacco mosaic virus by rubbing a suspension of virus on the leaves and immediately washing the excess virus from the leaves. Some of the inoculated leaves were harvested and frozen in moist chambers after five days, others after 20 days. Powdered K_2HPO_4 (3 per cent of leaf fresh weight) was added to the frozen leaves before grinding in a meat chopper. The juice was pressed out through cheesecloth and the virus was purified by the usual alternate centrifugations at 3000 r.p.m. and 35,000 r.p.m. The purification method differed from that of Spencer in that the solution used to suspend the virus pellets after ultracentrifugation was made by adding sufficient N/100 HCl to a 0.033 M solution of K_2HPO_4 to bring the pH to 7.2. Sorensen's

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Na_2HPO_4-KH_2PO_4 buffer (Clark, 1928) at pH 7 was substituted for the above phosphate-HCl solution in a single experiment indicated later.

It was sometimes possible to get 20-day virus sufficiently pure for microscopy after two ultracentrifugations. However, three ultracentrifugations were always required to purify 5-day virus. After the last ultracentrifugation, the virus pellet was always suspended in distilled water and various dilutions were than dried on the usual Parlodion membranes for microscopy in an R.C.A. type B electron microscope. Most of the micrographs were made at a magnification around 8000 and were then enlarged to around 30,000 before measuring the lengths of the virus particles with calipers. The magnification of the microscope changed frequently. It was, therefore, necessary to recalibrate the instrument from time to time in order to decrease the magnification error.

RESULTS.—The experimental work has involved the taking and enlarging of numerous electron micrographs and the measuring of the lengths of around 10,000 particles. In order to record the results in the available space, only a few of the micrographs can be included. A typical micrograph of 20-day virus is shown in figure 1. In general, the micrographs of 20-day virus were much better than those of 5-day virus. The reason for this difference in quality has not been determined.

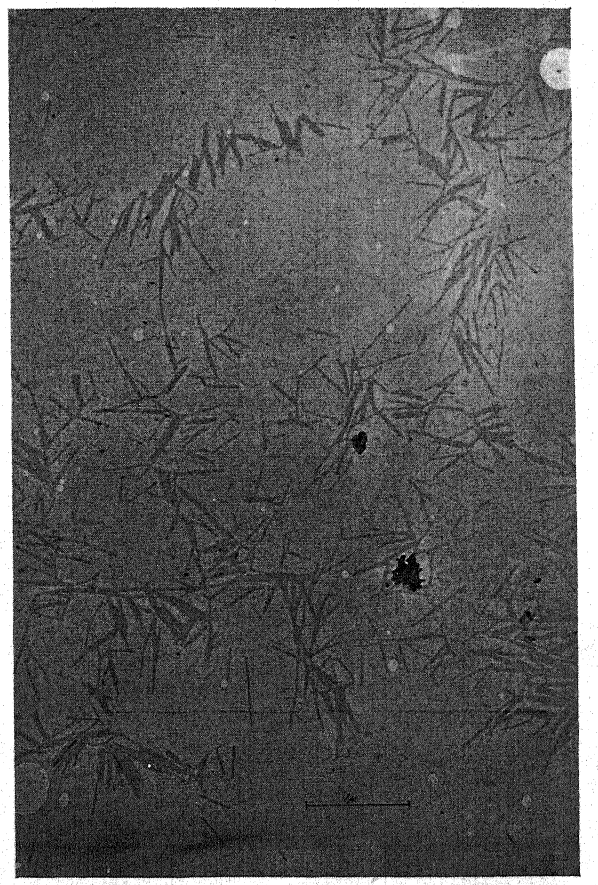


Fig. 1. Purified tobacco mosaic virus from plants infected 20 days.

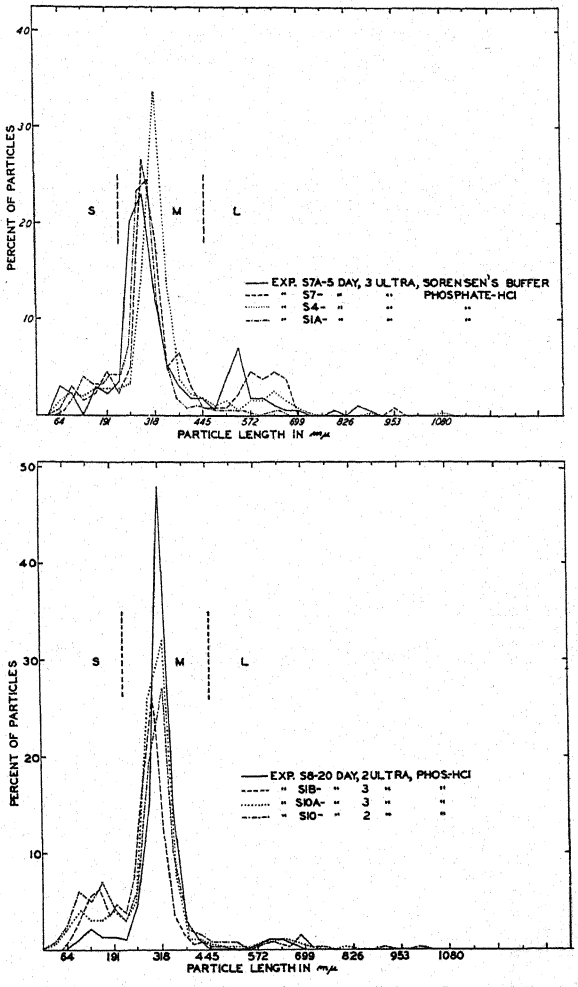


Fig. 2-3.—Fig. 2 (above). Length distribution curves showing length of 5-day virus particles purified by ultracentrifugation in phosphate-HCl solution and Sorensen's buffer.—Fig. 3 (below). Length distribution curves showing length of 20-day virus particles purified by ultracentrifugation in phosphate-HCl solution.

The lengths of the measured particles in the various experiments are shown in length distribution curves in figures 2 and 3. A further analysis of these curves is given in table 1. It is evident from the length distribution curves in figures 2 and 3 and from table 1 that, with the exception of the 5-day lot in Experiment S1A, the results confirm the sedimentation work of Spencer. In general, the 5-day lots contain a higher proportion of particles longer than 450 mμ than do the 20-day lots.

Three similar experiments were carried out with leaves that had been infected for two days. The purified preparations from 2-day leaves showed an even higher proportion of particles longer than 450

TABLE 1. *Length of 5-day and 20-day virus particles.*

Number of experiment and date	Age of infection in days	Number of ultra-centrifu-gations	Suspension medium	Number particles measured	Per cent under 225 mμ long	Per cent between 225 and 450 mμ long	Per cent longer than 450 mμ
S1A Nov., 1943	5	3	Phosphate-HCl solution	817	17	79	4
S4 Apr., 1944	5	3	Phosphate-HCl solution	735	11	76	13
S7 Dec., 1944	5	3	Phosphate-HCl solution	136	12	68	20
S7A Dec., 1944	5	3	Sorensen's phosphate buffer, pH 7	174	10	70	20
S1B Nov., 1943	20	3	Phosphate-HCl solution	599	26	73	1
S10A June, 1945	20	3	Phosphate-HCl solution	1173	16	79	5
S8 Feb., 1945	20	2	Phosphate-HCl solution	564	6	91	3
S10 June, 1945	20	2	Phosphate-HCl solution	828	25	70	5

mμ than did the 5-day leaves. However, five ultracentrifugations in phosphate-HCl solution were required to get 2-day virus pure enough for microscopy, and the results may therefore not be comparable with 5- and 20-day results.

It is evident from the length distribution curves in figures 2 and 3 that there are usually three rather distinct groups of particles which we shall for convenience of discussion call the S group (the short group made up of particles shorter than 225 mμ),

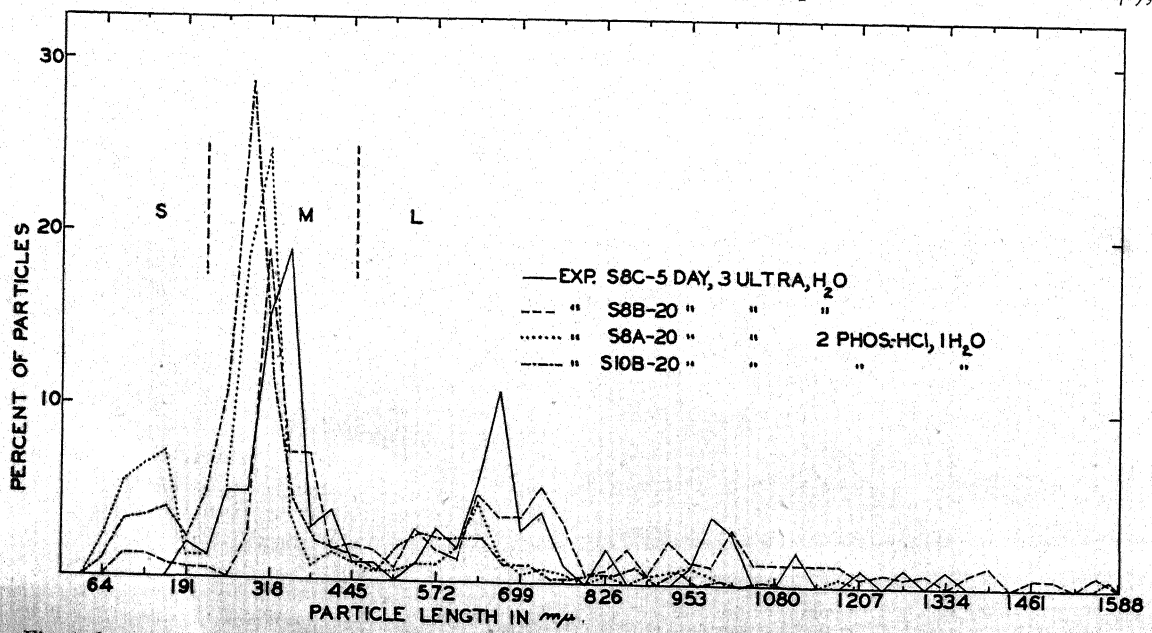


Fig. 4. Length distribution curves showing the end-to-end aggregation produced by ultracentrifugation in distilled water.

TABLE 2. *Relation of end-to-end aggregation of virus particles to method of purification.*

Number of experiment and date	Age of infection in days	Number of ultra-centrifu-gations	Suspension solution	Number particles measured	Per cent under 225 mμ long	Per cent between 225 and 450 mμ long	Per cent longer than 450 mμ
S10A June, 1945	20	3	Phosphate-HCl (Check)	1173	16	79	5
S8B Feb., 1945	20	3	Distilled H_2O	236	4	40	56
S8C Feb., 1945	5	3	Distilled H_2O	103	2	52	46
S8A Feb., 1945	20	2 in Phos.-HCl	Phosphate-HCl	518	25	58	17
		1 in distilled H_2O	Distilled H_2O				
S10B June, 1945	20	2 in Phos.-HCl	Phosphate-HCl	660	14	66	20
		1 in distilled H_2O	Distilled H_2O				

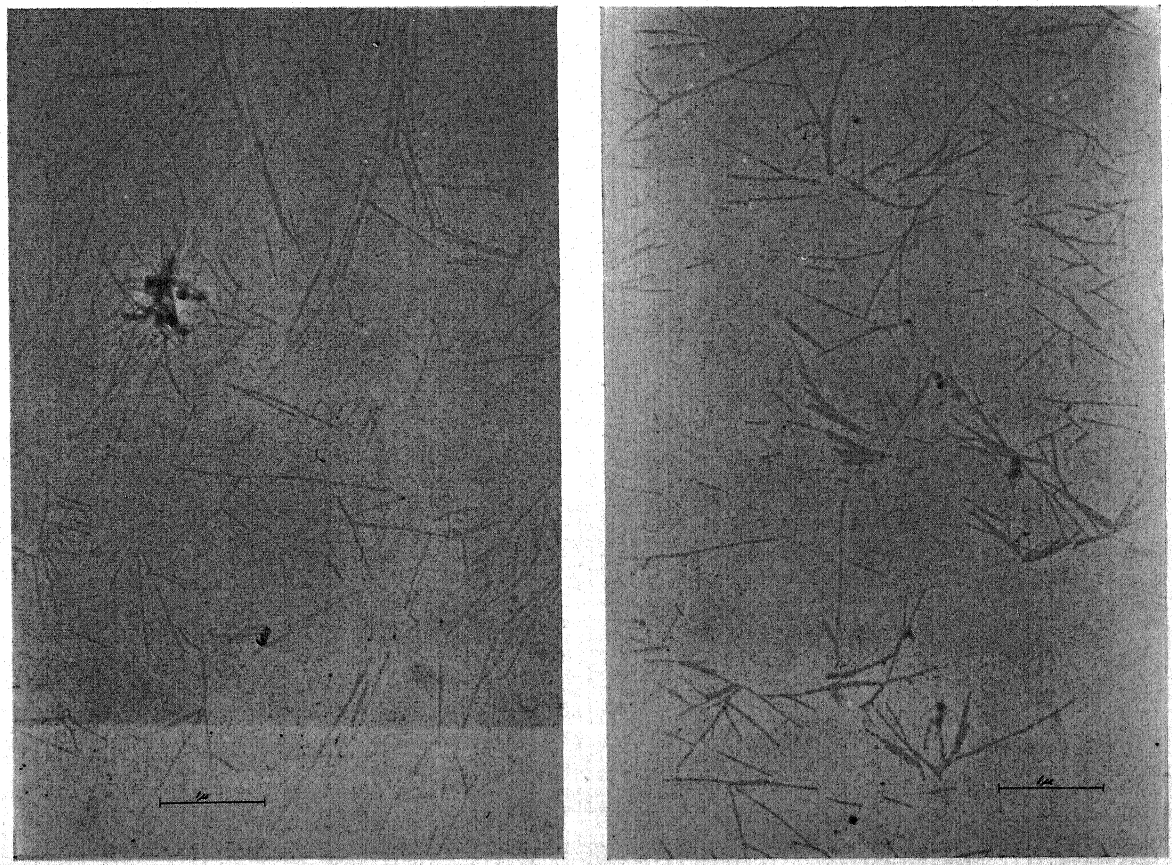
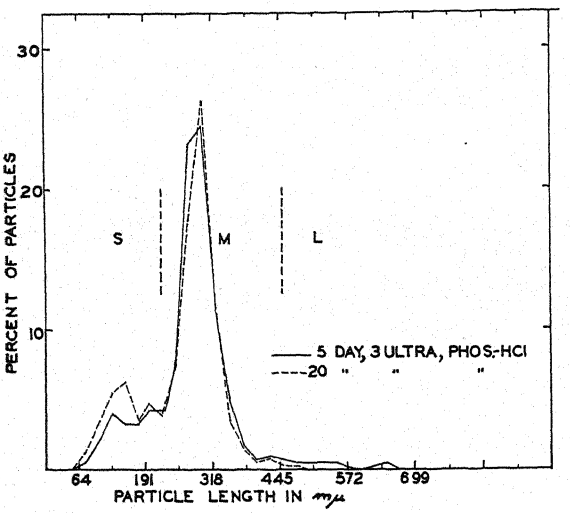


Fig. 5–6.—Fig. 5 (left). Apparent end-to-end aggregation of tobacco mosaic virus caused by 3 ultracentrifugations in distilled water.—Fig. 6 (right). Apparent end-to-end aggregation of tobacco mosaic virus caused by 1 ultracentrifugation in distilled water following 2 ultracentrifugations in phosphate-HCl solution.

the M group (the middle group composed of particles between 225 and 450 mμ long), and the long L group (composed of particles longer than 450 mμ).



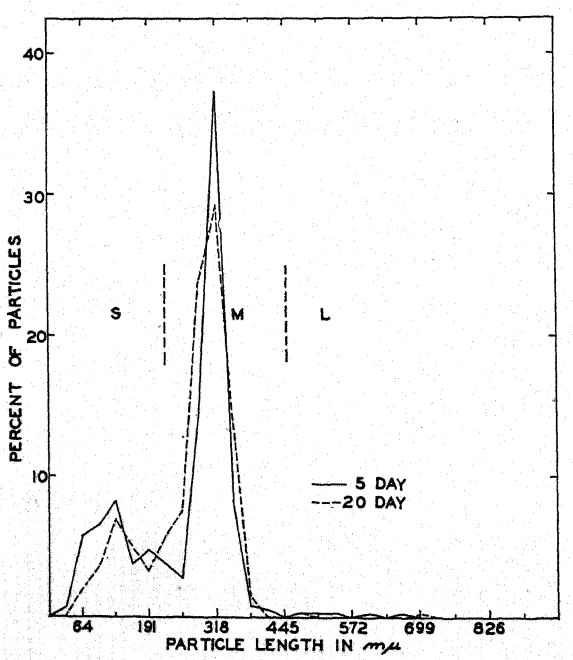


Fig. 7–8.—Fig. 7 (above). Length distribution curves showing length of 5- and 20-day virus particles before aging.—Fig. 8 (below). Length distribution curves showing length of 5- and 20-day virus particles after aging for 12 months.

The question arises as to whether the group of particles longer than 450 mμ found in the 5-day virus arose by gradual elongation of shorter particles while in the host, or by end-to-end aggregation of shorter particles before or during purification. An attempt was accordingly made to determine some of the factors that influence end-to-end aggregation during purification.

Experiments on aggregation during purification.—One 5-day sample and one 20-day sample were purified while suspended in distilled water without the addition of any powdered K_2HPO_4 or phosphate-HCl solution at any time during the process. Two 20-day samples were purified, after the usual powdered phosphate treatment, by two ultracentrifugations in phosphate-HCl solution, followed by a third ultracentrifugation in distilled water. The marked end-to-end aggregation caused by ultracentrifugation of virus suspended in distilled water is shown in the length distribution curves in figure 4 and in table 2. The greatest amount of end-to-end aggregation occurred when the virus was suspended throughout in distilled water, but a significant amount occurred after a single ultracentrifugation in distilled water following two ultracentrifugations in phosphate-HCl solution. Typical micrographs showing the results of these treatments are shown in figures 5 and 6.

Since there is a good possibility that the low pH of the virus suspension in distilled water may be one of the important factors in causing end-to-end aggregation during ultracentrifugation in distilled water, the question arises as to whether the juice extracted from 5-day infected leaves may have a lower pH than that from 20-day leaves and that the particles longer than 450 mμ in 5-day leaves may therefore be explained as due to end-to-end aggregation before centrifugation. Determinations of the pH of juice extracted from frozen 5 and 20-day infected leaves from several plants failed to show any indication of a lower pH in 5-day juice. In fact, the 20-day juice tended to show a lower pH than did the 5-day juice. It therefore appears that we cannot explain the longer particles in 5-day virus ultracentrifuged in phosphate-HCl solution as due to aggregation caused by initial low pH. If these particles are formed by end-to-end aggregation, some undetermined factor or factors must be involved.

Our results with distilled water apparently do not agree with those of Wyckoff (1937) and those of Lauffer (1944), who reported least aggregation of virus when purified by ultracentrifugation in distilled water. Our results are in agreement with those usually observed with proteins. In general, proteins show increasing aggregation as the pH approaches the isoelectric point. The isoelectric point of tobacco mosaic virus is between pH 3 and 4. The pH of a distilled water suspension of virus is ordinarily between 5 and 6 as contrasted to phosphate-HCl solution having a pH of 7.2. A low concentration of salts has regularly been found to be less favorable for aggregation of proteins than is a salt-free solution of proteins. It therefore appears that a dilute phosphate-HCl solution at pH around 7 would be expected to produce less aggregation of a proteinaceous virus than does distilled water.

Experiments on aging.—After standing in a refrigerator at 1°C. for 12 months, a 5- and a 20-day virus sample, purified by three ultracentrifugations in phosphate-HCl solution, were micrographed a second time. The length distribution curves of the 5- and 20-day virus before and after aging are shown in figures 7 and 8. These results do not show any evidence of end-to-end aggregation of purified virus while simply standing at low temperature in distilled water. It, therefore, appears that ultracentrifugation is an important factor in causing aggregation when a virus is suspended in distilled water.

Reduction of errors.—The fact that a very high proportion of the measurable virus particles usually have lengths so close to 300 mμ brings up the question as to whether the group of particles having lengths around 300 mμ (the M group) may be composed of particles, all of which are of the same length, and whether the variation observed within the group may be due to various errors involved in obtaining the results. If this variation can be explained by the errors involved, the results furnish strong evidence for Stanley's (1938) theory that the particles of certain viruses are macromolecules, since all molecules of a particular organic compound usually have the same size.

There are several magnification errors involved in measuring virus particles. According to Dr. A. M. Cravath of the Shell Development Company, Emeryville, California, the magnification of particles at the margin of our pictures may differ as much as 13 per cent from that in the center of the picture. The particles having the long axis in one direction may be magnified as much as 3 per cent more or less than the particles having the long axis perpendicular to this direction. The change in magnification from one micrograph to another (error x) may be as much as 2 per cent. A fourth error is in measuring a particle. We have found by measuring a single particle numerous times with calipers that one may get results differing as much as 7 mμ. In order to avoid or reduce these errors, several of our best micrographs were selected. The particles in a 2-inch square in the middle of these enlarged micrographs were measured. All of the particles selected for measurement had lengths between 250 and 350 mμ. The lengths of the measured particles within a single square were compared. By this method error x was eliminated, and the error due to location on the micrograph was reduced to 0.4 per cent. The 3 per cent error due to direction of the particles and the 7 mμ error of measurement remained unchanged. By adding these errors, it appears that the total error is around 17.2 mμ. Any difference in the length of the measured particles must therefore be greater than 17.2 mμ to be considered significant. The results of the measurements are shown in table 3.

It is evident from these results that there are often particles of the M group within a 2-inch square in a single picture that differ from each other in length by more than 17.2 mμ. It therefore appears reasonable to conclude that the M group is not composed of particles having exactly the same length.

TABLE 3. *Variation in length of particles in M group within a 2-inch square of single micrographs.*

Micrograph number	Length of particles in mμ
156-2A	311, 333, 305
193-3B	335, 308, 305, 312, 308
193-1C	312, 322, 318, 265, 328, 299, 325, 308, 265
193-4A	348, 335, 315, 342, 295
192-5A	272, 308, 318, 318, 342
193-2B	308, 308, 299, 289, 289, 308, 282, 265
156-4A	305, 321, 314, 311, 295, 295, 311, 305, 327, 305

DISCUSSION.—The group of particles shorter than 225 mμ (S group) was first observed and discussed by Stanley and Anderson (1941); no conclusions were reached regarding their origin. They were discussed further by Frampton (1942) and by Rawlins (1942), but their origin and nature have never been satisfactorily explained.

According to Bawden (1945) the residue of the infected leaf, remaining after juice has been extracted, contains a high concentration of active tobacco mosaic virus particles that are considerably shorter than those found in purified virus obtained from leaf juice. It is, therefore, possible that our S group of particles may represent a portion of the group reported by Bawden.

Although the S group is regularly found in micrographs, it has never been reported in the numerous publications on the sedimentation constant of tobacco mosaic virus. This evidence may indicate that the S group is absent in purified virus suspensions prepared from plant juice and may be formed by transverse fracture of longer particles during drying on the Parlodion membrane.

It is evident that more information must be obtained before conclusions can be reached regarding the origin of the S group and the relation of this group to the M group.

The middle (M) group is the most conspicuous group of particles in the length distribution curves. It regularly contains the highest proportion of virus particles. In one experiment (table 1) in which 20-day virus was purified by two ultracentrifugations in phosphate-HCl solution, the M group contained 91 per cent of the measurable particles. The smallest proportion (40 per cent) of particles was found in this group when the virus was purified while suspended throughout in distilled water (table 2). The low proportion in the M group was here apparently due to end-to-end aggregation of particles in this group to form long particles in the L group.

Bawden (1945) has put forth the idea that the virus particles found in infective leaf juice have been produced by end-to-end aggregation of shorter

virus particles. This does not appear to us to be a satisfactory explanation of the origin of the prevalent M group. It appears unlikely that short detectable virus particles would so regularly stop aggregation when they have reached a length around 300 mμ. We believe that the particle around 300 mμ is prevalent in the host and that there is as yet no good evidence as to its mode of formation.

The origin of the L group is rather obvious in the experiments where the virus was suspended in distilled water during purification. Our experiments appear to offer good evidence that here a large proportion of the particles in the L group is formed by end-to-end aggregation during purification. The mode of formation of the L group in 5-day virus purified while suspended in phosphate-HCl solution remains uncertain. The ease with which double length particles can be produced by end-to-end aggregation during purification in distilled water favors the idea that the double length particles in 5-day virus purified in phosphate-HCl solution may have been formed by end-to-end aggregation.

If the particles in the L group were formed in the host by the gradual elongation of particles in the M group, there should be intermediate lengths between the two groups, particularly in the 5-day virus where multiplication is rapid. Such particles should decrease the slope on the right side of the M group. An examination of figure 2 fails to show any clear evidence of a more gradual slope on the right side of the M group than on the left side of this group. This result is further evidence that end-to-end aggregation accounts for the formation of the L group of the 5-day virus.

On the other hand there is no evidence from pH studies to indicate that conditions in 5-day virus were more favorable for end-to-end aggregation than were conditions in 20-day virus.

The high proportion of particles longer than 450 mμ in 5-day virus may explain the results of Stanley and of Spencer in which they found fewer local lesions produced by a given weight of purified 7- and 5-day virus than by the same weight of 28- and 20-day virus.

Considerable side-to-side and side-to-end grouping of particles often occurs as seen in figure 1. No evidence of a relation of age of infection to side-to-end aggregation was detectable in our micrographs. No satisfactory method has been found for accurately determining the amount of side-to-side grouping in the various micrographs. A difficulty in interpreting the results is the uncertainty as to whether various types of aggregation occurred before or during the drying of the virus preparation on the Parlodion membrane. Sedimentation studies (Wyckoff, 1937; Spencer, 1942; Lauffer, 1944) that have shown more than one advancing boundary have usually shown two boundaries, one due to the M group and a second due to a group of larger particles. If it is assumed that the second boundary is caused by elongated particles that have arisen by end-to-end aggregation, as Lauffer's (1944) results indicate, it is reasonable to suppose that the side-to-end and side-to-side aggregations were not present before drying when sedimentation studies were made, but resulted during drying of the virus on the Parlodion membrane. The fact that 20-day virus preparations that have particles very uniform in length, and presumably producing a single boundary during sedimentation, show much side-to-side and side-to-end aggregation when micrographed is further evidence that these types of aggregation occurred during drying of the virus.

SUMMARY

Electron microscope studies confirm Spencer's sedimentation studies indicating that purified tobacco mosaic virus preparations from leaves infected for five days usually contain a higher proportion of double-length particles than do similar virus preparations from leaves infected 20 days. It has not been possible to prove whether the double length particles resulted from gradual elongation of virus particles while in the host or from end-to-end aggregation of two typical particles having a length around 300 mμ. Most of the evidence apparently favors the latter explanation.

The prevalence of particles longer than 450 mμ in 5-day virus may explain the results of Stanley and of Spencer in which they found fewer local lesions produced by a given weight of purified 7- and 5-day virus than by the same weight of 28- and 20-day virus.

Our results indicate that purification by alternate high and low speed centrifugation while the virus is suspended in dilute phosphate-HCl solution causes less end-to-end aggregation than similar purification of virus suspended in distilled water.

A very high proportion of tobacco mosaic virus particles regularly have a length around 300 mμ. The length of these particles has apparently been very accurately controlled by some factors which have kept the length within narrow limits but have not produced particles of exactly the same length. All of the available evidence indicates that particles of this length were present in the host plant. It does not appear probable that these particles, showing so little variation in length, have been formed by the end-to-end aggregation of shorter virus particles.

Storage of purified virus around 12 months in distilled water at 1°C. failed to cause detectable aggregation of particles.

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EFFECT OF OXYGEN CONCENTRATION ON RESPIRATION OF THE FUERTE AVOCADO FRUIT [1]

J. B. Biale

INVESTIGATORS IN the field of fruit physiology and storage have devoted most of their attention to the effects of combined changes in the oxygen and carbon dioxide content of the atmosphere. This approach was prompted by the need to outline the best conditions for modified air storage. Little information is available on the response of fruit to variations in the oxygen concentration. Parija (1928), working with apples, found the lowest CO_2 production at 5 per cent O_2; it rose to higher levels below and above this critical concentration. Kidd and West (1934) succeeded in retarding the onset of the climacteric rise in apples by 5 per cent O_2 and in accelerating it by pure oxygen if the response to air was used as control. Singh (1937) found the critical oxygen concentration for mangoes to be 9.2 per cent. In some of these studies the anaerobic CO_2 production was found to be high over a considerable period of time, indicating the capacity of the tissue for making use of the fermentative enzyme system. In actively growing plant parts CO_2 evolution in pure nitrogen was very low as compared with mature fruits.

The avocado fruit occupies a unique position with respect to the relationship between the aerobic and the anaerobic process. Wardlaw and Leonard (1935) were able to depress respiration and delay maturation in some West Indian varieties of avocados by reducing the oxygen concentration and increasing the carbon dioxide level. However, their measurements were too few to illustrate the changes in the course of respiration of fruit subjected to a modified atmosphere. Biale (1941) found that in air the respiratory activity of Fuerte avocados at 15°C. was characterized by a rapid acceleration in CO_2 production followed by a decrease. This so-called climacteric rise did not take place in an atmosphere of nitrogen, nor did the fruit ever reach the soft, edible stage. From the standpoint of fruit storage, the question arose whether the physiological changes characteristic of the climacteric rise could be delayed without altering the nature of the respiration process. It was hoped that the onset of the climacteric and its actual value could be controlled by changes in oxygen tension. The effects of variation of oxygen concentration at different temperatures were studied in the experiments reported here.

MATERIALS AND METHODS.—The avocados for these experiments were of the Fuerte variety picked from 6 to 7 year old trees in the Horticulture orchard of the College of Agriculture, Los Angeles campus of the University of California. In the climatic zone in which this orchard is situated, the Fuerte variety normally attains horticultural maturity in December and may be harvested through the month of June. Size was the chief criterion employed in selecting uniform fruit since variability in the respiration behavior of fruits from one tree appeared to be as great as between specimens of different trees. When the carbon dioxide evolution of individual avocados was determined, it was found that the differences in time of the climacteric maximum were of the order of ten days. In the case of a composite sample the peak coincided with the earliest of the single fruits, and variabilities between different lots were much smaller. Hence each sample consisted of twenty avocados with the exception of experiments 8 and 13 in which 15 and 21 fruits were used respectively. The range in average fruit weight for samples of twenty Fuerte avocados was from 4552 grams in experiment 3 to 5964 grams in experiment 14. The variability was much lower for the several jars in each experiment.

For the studies of the effects of the oxygen variable, gas mixtures were prepared by combining the proper proportions of air and nitrogen for subatmospheric oxygen concentrations, and by mixing pure oxygen with nitrogen for oxygen tensions above those in air. The gas mixing board (fig. 1) consisted in its essential parts of carefully calibrated flowmeters, needle valves, and a mixing cylinder 18 inches long and 2½ inches in diameter. Air was delivered from a constant pressure compressed air supply line by means of a needle valve to the mixing board, where another needle valve and an air bleeding device made it possible to maintain

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TABLE 1. *The actual oxygen concentrations obtained from the gas mixtures.*

Per cent O_2 reported	Per cent O_2 found by gas analyses							
	Exp. 3	4	6	7	8	9	12	13
2.5	2.25	2.54	2.52	...	2.59	...	2.62	2.48
5.0	5.00	5.35	5.13	4.73	4.91	5.07	5.01	5.35
10.0	9.81	10.37	10.29	9.58	9.85	9.83	9.38	9.95

reasonably constant flow rates. The oxygen and nitrogen cylinders were equipped with accurate pressure regulators which allowed for a constant gas supply of low pressure (3 to 5 lbs.) throughout the range of pressures in the cylinders. Here again the use of additional needle valves on the board facilitated fine adjustment of the flow. The flowmeters were calibrated by the method of Meuron (1941), displacing through them air from a 50 or 250 ml. burette which was being filled at different rates with water from a source of constant hydrostatic pressure. Calibration curves were prepared in which rates of flow in milliliters per minute (ml/min) were plotted against flowmeter deflections. The maximum error in oxygen concentration due to deviations in flowmeter readings was of the order of 1 per cent. Since the respective gases were delivered through flowmeters, the oxygen concentrations reported were on a volume percentage basis. The composition of the modified atmospheres was checked periodically by the Orsat method, and the results of the gas analyses for most of the experiments described here are given in table 1.

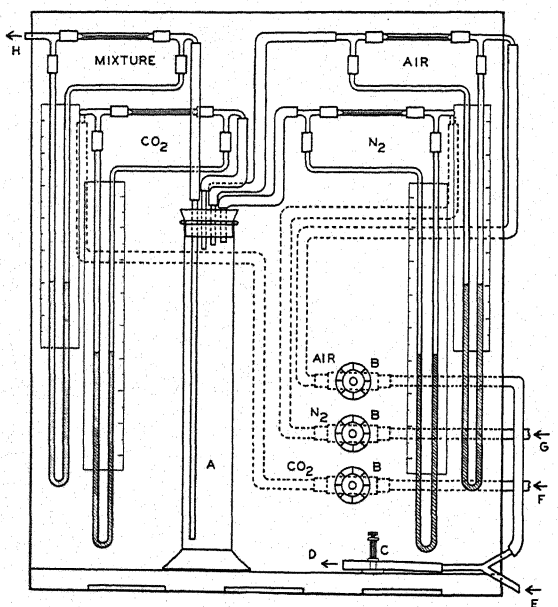


Fig. 1. Apparatus for preparing gas mixtures. 1/8 of original dimensions. A, mixing cylinder; B, 1/8 inch needle valves; C, screw clamps; D, outlet for excess air flow; E, from compressed air line; F, from CO_2 cylinder; G, from nitrogen cylinder; H, to respiration jar.

The figures given in the first vertical column will be used in this report for the sake of convenience, but reference may be made to this table for the actual compositions of the modified atmospheres. The percentage of oxygen in air, which was always used as the control, was found at different times to be approximately 20.8. The above indicated oxygen concentrations were employed at the following temperatures: 15, 10, 7.5, and 5°C. In experiments 7 and 8 at 15°C. the fruit was subjected to a wider range of oxygen concentrations, the analyses of which will be included below.

The method of determining CO_2 production was a modification of the procedure used by Claypool (1938) and consisted of passing air freed of carbon dioxide through the specially constructed respiration jar described by Biale and Shepherd (1941) and into a sintered glass bubbler immersed in a sodium hydroxide solution in a hydrometer cylinder. The quantity of 0.3 N base used varied from 25 to 75 ml., depending on the intensity of respiration. After a suitable period of time, usually 30 minutes for avocados, 10 ml. of 1.5 N $BaCl_2$ were added for each 25 ml. of base used, and the excess alkali titrated with 0.15 N hydrochloric acid to a thymolphthalein end point. This procedure will be referred to as method I to distinguish it from method II of measuring CO_2 evolution which was used in connection with the determination of oxygen absorption, and which will be described below.

The rate at which the gas mixtures were passed over the fruit had to be high enough to prevent significant changes in the composition of the atmos-

TABLE 2. *Effect of air rate on the respiratory course of Fuerte avocados.*

Jar number	Air rate ml/min	Mg CO_2 per kg-hour			Number of days from initial to	
		Initial	Minimum	Maximum	Minimum	Maximum
12	200	65.6	35.3	126.0	8	12
13	300	66.0	32.7	124.9	5	12
14	400	69.3	33.4	134.0	5	12
22	500	64.2	33.5	127.0	7	12
24	600	71.2	32.2	114.9	7	14

phere within the respiration jars. Two experiments were designed to determine the non-limiting rate of flow. In experiment 1, two jars with twenty fruits in each jar were placed at 15°C. under an air rate of 350 ml/min. The initial difference in carbon dioxide production between the two samples was less than 2 per cent. One of these samples was constantly subjected to the rate of 350 ml/min, while in the case of the second jar daily increases in the rate of flow of 50 ml/min were introduced until a rate of 500 ml/min was attained. Respiration determinations were carried out before and after the changes in flow. The results obtained indicated that there was an immediate rise in CO_2 evolution following increase in flow, but after one hour equilibrium was established and the rate of CO_2 evolution did not differ materially from that of the control jar which was maintained constantly at 350 ml/min.

In a second test on rates of flow (experiment 2), five composite samples picked on February 25 were placed at 15°C. under five different rates of flow. The course of respiration was followed until the post-climacteric stage; the results are given in table 2.

The uniform behavior of the several samples is evident from the results in this table. Close parallelism was observed also in the softening process in the five jars. On March 9 (12 days after picking), all the avocados were firm with the exception of several fruit that had barely started softening. Four days later most of the fruit in each jar were in the edible stage. It appears from this test that with respiration intensities occurring in air at 15°, a flow of 200 ml/min was sufficient to prevent excessive accumulation of CO_2 and significant depletion of oxygen in the jar. Since, however, larger respiration rates than those in air were expected with fruit subjected to 50 and 100 per cent O_2, it was decided to standardize on a rate of flow of 350 ml/min. With this rate, three jars could be treated simultaneously from each mixing board. Considering a peak value of 150 mg CO_2/kg-hour, the concentration of CO_2 in the container at equilibrium should not exceed 1.8 per cent at an air rate of 350 ml/min. No tests were made with rates lower than 200 ml/min because of previous findings with lemons, which have a much lower respiration intensity than avocados, that lower rates affect the CO_2 evolution of the material (Biale and Shepherd, 1941).

EXPERIMENTAL RESULTS.—*Effect of oxygen concentration on carbon dioxide production at 15°C.*—The decision to study extensively the various aspects of the respiratory activity of the avocado at 15°C. was based on observations made at temperatures varying from 5°C. to 25°C. The physiological changes characteristic of the senescent stage proceed at 15°C. at a rate at which they can be observed readily. They are neither too rapid nor too slow as compared with higher and lower temperatures. When, for example, fruit is placed under constant environmental conditions immediately after picking, there results a decrease in the rate of CO_2 evolution which may last for several days. This preclimacteric behavior is seldom observed at 25°C. On the other hand, at 5°C. the rise in respiration may escape detection because of limitation in the method of measuring low levels of CO_2 production. Therefore, most of the results in this study were obtained from tests at 15°C. conducted during the 1942–43 and 1943–44 seasons. Six representative experiments (numbers 3 to 8, inclusive) will be summarized here with each of these tests illustrating different aspects of the problem of oxygen tension effects.

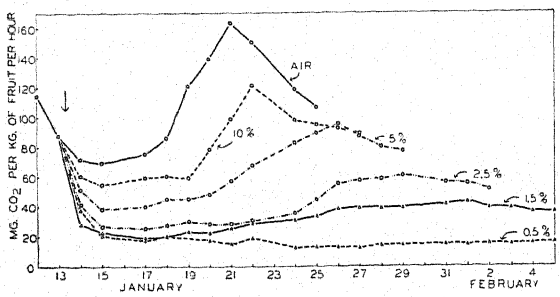
The avocados for experiment 3 were picked in the morning of April 19, 1943, divided into four lots, and placed the same day in a controlled room maintained at 15°C. ± 0.5°C. Though the variability in fruit weight of these samples was considerably higher than that in the later experiments, the initial respiration values taken in air and based on fresh weight were reasonably uniform. On April 20 the three semi-hourly respiration measurements gave rates of CO_2 evolution that differed by 2 per cent from the mean. In view of the uniformity in response of this material, the several jars were subjected to differential treatment on April 20 immediately after the respiration determinations. The effect of the different oxygen partial pressures was immediately observable, as shown in table 3.

TABLE 3. *Effect of oxygen tension on CO_2 evolution by Fuerte avocados (CO_2 in mg per kg of fresh weight per hour).*

Jar number	Treatment	April 20	Treatment % O_2	21	22	24	26	27	28	29	30
3	air	72.9	2.5	25.5	21.1	17.3	19.0	21.8	24.7	31.9	34.6
6	air	73.2	5.0	31.9	27.2	29.5	29.3	37.4	41.7	65.7	72.4
7	air	71.2	10.0	38.1	34.7	31.9	45.7	84.2	86.3	100.6	87.7
30	air	69.2	air	52.6	48.9	80.2	118.7	126.2	115.9	115.0	105.6

Jar number	% O_2	May 1	2	3	4	5	6	7	8	10	
3	2.5	44.6	44.0	48.0	48.8	47.0	50.5	42.7	40.1	37.7	...
6	5.0	81.4	73.6	66.7	61.3	63.3	57.3	50.6	46.9	41.1	...
7	10.0	64.6	51.6	62.0	61.4	...	...	56.5	54.5	50.7	...
30	air	88.6	82.7	78.9	66.4	69.7	70.0	64.4	60.1	60.0	...

All of these results are averages of three semi-hourly measurements. The readings on April 21 were taken 21 hours after the change from air to the several oxygen concentrations. At that time the CO_2 production in jar 3 under 2.5 per cent O_2 was 48.5 per cent of the respiration rate of control jar



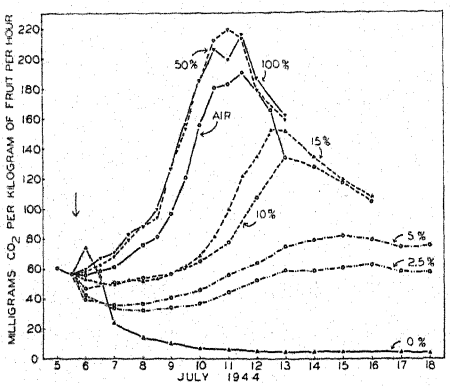


Fig. 2 (above). Effect of oxygen (0.5 per cent to air) on CO_2 production of Fuerte avocados at 15°C. (Arrow indicates beginning of differential treatment.)—Fig. 3 (below). Effect of oxygen (0 to 100 per cent) on CO_2 production of Fuerte avocados at 15°C. (Arrow indicates beginning of differential treatment.)

30. In all cases there was a characteristic decrease as compared with the values obtained immediately after harvesting. The pre-climacteric minimum was followed by a sharp increase to a climacteric peak. The relative respiration values and the number of days from the pre-climacteric minimum to the peak are presented in table 4.

TABLE 4. *Relative respiration values and duration of the climacteric rise.*

Jar number	Treatment % O_2	Relative CO_2 production minimum	maximum	No. of days minimum to peak
3	2.5	35	40	12
6	5.0	56	64	9
7	10.0	65	80	5
30	air	100	100	5

The post-climacteric period was characterized by a pronounced decline in CO_2 production accompanied by fruit softening. The chemical transformations that are responsible for the change in the firmness of the avocado flesh probably were set into motion before the peak was reached, but observable softening did not take place until after the maximum was attained.

Since softening is a function of the climacteric rise, there were marked differences between the several treatments in experiment 3 with regard to the storage life of the fruit. On April 30 all the avocados in jar 30 (air) were softening, while in jar 7 (10 per cent O_2) eight had barely started to soften, with the remainder still firm or hard. On May 2 when the control fruit was soft, in jar 7 there were five avocados in the nearly edible stage, eleven softening and four firm. In jar 6 (5 per cent O_2) eleven had barely started to soften, and nine were still firm. On that day the avocados in jar 3 (2.5 per cent O_2) had hardly changed in their texture, but on May 4 eight fruit began to soften, and three days later most were in the edible stage. On May 10 when the experiment was concluded, the fruit in jars 3 and 6 had the best appearance, while a good deal of mold was observed in the control jar and somewhat less in the jar subjected to 10 per cent O_2. Since the total duration of this experiment was twenty days, it is reasonable to conclude that the reduced oxygen content of 2.5 and 5 per cent had doubled the storage period of the fruit.

The end of the storage life of a sample of avocados is difficult to determine with precision. The best edible stage may vary from individual to individual. An objective measure of comparison might be arrived at by counting the number of days from the date of picking to the climacteric peak, as illustrated in table 5.

TABLE 5. *Number of days from picking to climacteric peak under different oxygen tensions.*

Exp. number	Date of picking	Per cent oxygen 2.5	5.0	10.0	Air
3	4–19–43	17	12	10	8
6	6–16–43	10	10	7	7
7	1–11–44	18	15	11	10
8	7– 4–44	12	10	9	7

It appears from a comparison of the early season experiment 7 with the late season experiment 8 that the time interval under consideration may be a function of fruit maturity. With the exception of experiment 6 the time lapse between picking and climacteric peak was nearly twice as long in the lowest oxygen treatment as compared to air. This relation was borne out by other experiments not reported here.

The general relationship between CO_2 production and the oxygen content of the storage atmosphere, as described above, was found to hold consistently in all the tests conducted throughout the picking season of the Fuerte variety. In some experiments a wider range of oxygen concentration was selected. In experiment 7 (fig. 2) the oxygen

tension varied from 0.5 per cent to 20.8 per cent in air. Besides the treatments indicated in this figure, 1 per cent and 2 per cent oxygen were also employed, giving results intermediate between 0.5 per cent and 2.5 per cent O_2. The exact analyses of the gas mixtures included in this experiment and not shown in table 1 are 0.60, 1.25, 1.59, and 2.06 volume per cent for jars 9, 10, 14, and 16, respectively. The avocados for this experiment were picked on January 11, and placed the same day at 15°C. The weights of the samples varied from 5050 to 5295 grams, or a variation of 2.5 per cent from the mean. For the first two days all jars were under air. Because of close agreement in CO_2 evolution for the 8 jars, the averages only are presented for January 12 and 13. The first striking feature of these results is the initial high value of CO_2 production following picking. The magnitude of the initial rate may be ascribed to a respiratory condition of the fruit on the tree, the effects of which are carried over after picking. It is unlikely that it could be related to environmental factors, such as the temperature in the orchard at harvesting, because it was in the month of January that the initial rates were twice as high as in July. They varied from an average of 110.5 for experiment 7 to an average of 61.4 for experiment 8. To obtain a clearer insight into this problem it would seem advisable to follow the respiratory behavior of the fruit prior to, as well as following, harvesting.

Differential treatment for experiment 7 was started on January 13. The rate of CO_2 evolution kept on declining in all cases until a preclimacteric minimum was reached which varied for the different oxygen concentrations. The magnitude of this minimum appeared to be more constant throughout the season than the initial rates. If we take as an example the control jars, the minimum respiration rate fluctuated from 48.1 in experiment 5 (5/29/43) to 69.1 in experiment 7 (1/15/44). For 5 per cent O_2 this value varied from 27.2 to 36.2 mg CO_2 per kg-hour. When the lowest rates of CO_2 production are considered, it was found that they bear about the same quantitative relation to oxygen tension as do the climacteric maxima. In the case of 0.5 per cent O_2, the increase was so gradual that one could not be sure that it was beyond experimental error. For all the other oxygen concentrations the peak respiration rates varied from 33.1 for 1.0 per cent O_2 to 163.0 for air, and the comparable interval between picking time and the peak was 18 days in the former and 10 days in the latter. As far as fruit softening is concerned, this experiment confirmed the previous one for the four highest oxygen concentrations. In the container subjected to 2 per cent O_2 only half of the fruit were edible on February 8, when the experiment was completed. The percentage of soft fruit was even smaller in the lower oxygen treatments.

In order to obtain a more complete picture of oxygen effects on avocado respiration, experiment 8 was designed to include oxygen concentrations higher than air. The results of this experiment are shown in figure 3, which also includes the respiration trends under previously employed conditions as well as the response to 100, 50, and 15 per cent O_2. Two jars were used for each of these new oxygen concentrations. The gas analysis of the pure

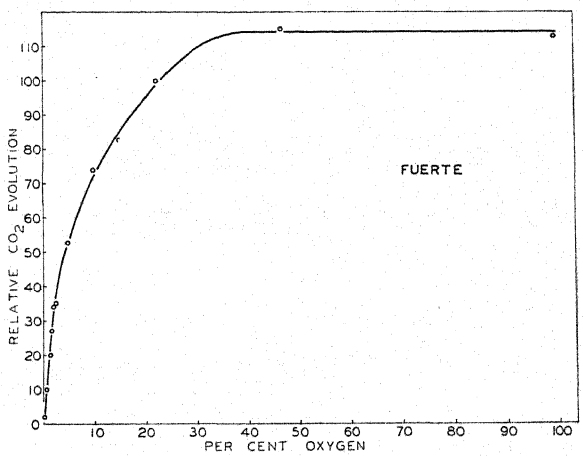
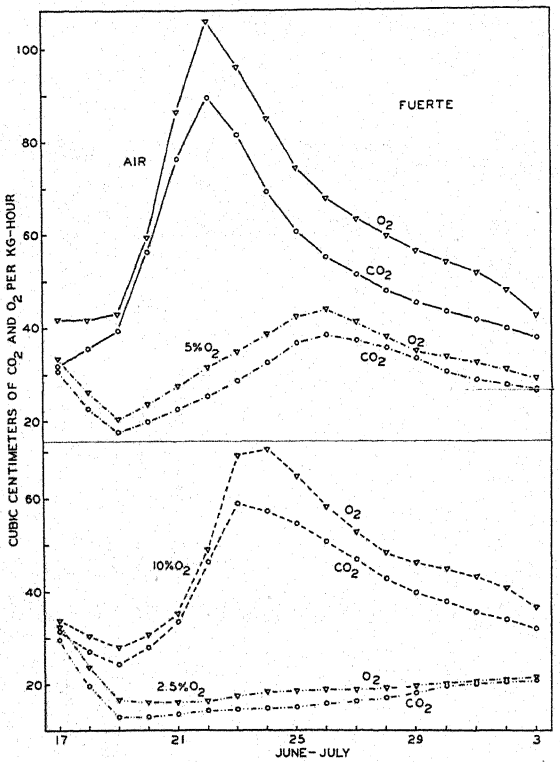


Fig. 4. Relative respiration rates of Fuerte avocados for the climacteric peak at 15°C. in relation to oxygen concentration. (Air value equals 100.)

oxygen cylinder showed 99.42 per cent O_2, the 50 per cent O_2 was actually 46.68 per cent and the 15 per cent amounted to 14.76 per cent. Because of rapid changes at high oxygen levels, the respiration determinations were carried on twice each day. In this test there were only 15 fruit per jar weighing from 3905 to 4110 grams per sample with a deviation of ± 2.5 per cent from the mean. Since the material for this experiment consisted of late season fruit, the initial rates were a good deal lower than in experiment 7, and consequently the decrease in the rate of respiration from the initial to the minimum was less pronounced. The respiratory activities of the 50 per cent and 100 per cent oxygen samples were characterized by an immediate rise after differential treatment commenced. The rates for the two highest concentrations were almost identical, and the peak value was slightly but not significantly higher in 50 per cent than in 100 per cent oxygen. Neither of these treatments differed greatly from air. The climacteric peaks took place on the same day, and the fruit softening was only slightly delayed in air as compared with 50 per cent and 100 per cent O_2. On the ninth day, July 14, all the fruit in the 50 and 100 per cent oxygen jars were soft, edible, and in excellent condition, while in the air jar there were four avocados which were not quite edible. The behavior of the avocados under 2.5 per cent, 5.0 per cent, and 10 per cent O_2 was not much different from that in previous experiments. In 15 per cent oxygen the fruit response with respect to softening as well as respiration was intermediate between air and 10 per cent O_2.

It is of interest to examine briefly the carbon

dioxide production of jar 8, which was kept in pure nitrogen freed of residual oxygen by passage over hot copper. An immediate rise in CO_2 evolution was followed by a sharp decline until the rate leveled off to a value of about 5 mg CO_2 per kg-hour. After 8 days, on July 13, a putrid odor emanated from this jar. After 14 days (on July 19) the fruit was transferred to air, resulting in a 3-fold increase in CO_2 production. The fruit rapidly turned brown, became subject to attack by fungi, and it never softened. Clearly, the avocado is highly sensitive to anaerobic conditions.



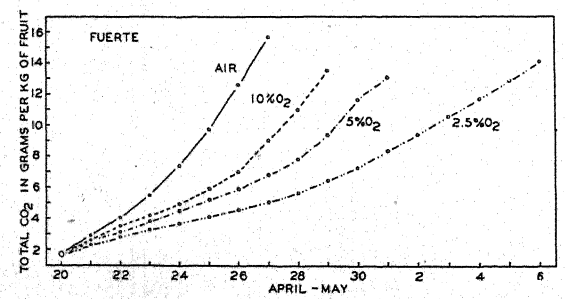


Fig. 5 (above). Simultaneous measurements of oxygen absorption and carbon dioxide evolution of Fuerte avocados at 15°C. in relation to oxygen tension.—Fig. 6 (below). Cumulative carbon dioxide production by Fuerte avocados at 15°C. as related to oxygen tension. Initial to climacteric peak values plotted.

In order to relate respiration rates to oxygen tension, the peak values of CO_2 production were selected from the several experiments and plotted against volume per cent of O_2 (fig. 4). Respiration in air for each test was taken arbitrarily as 100. For the anaerobic condition, the CO_2 value chosen was the one arrived at after the rate leveled off, since no climacteric rise took place in nitrogen. If that value were obtained within twelve hours or even one day after transfer from air to nitrogen, the curve would have assumed a different shape. We would observe then a critical oxygen concentration of 0.5 or 1.5 at which the CO_2 evolution is at a minimum. Before a final decision is reached on this point it may be necessary to find out what time period must be allowed for the change from one environmental condition to the next one. If this interval is prolonged, certain toxic substances may accumulate due to the absence of oxygen and suppress the CO_2 evolution below the rate characteristic for the anaerobic process. Insufficient data are available to indicate whether this was the case with the material under consideration, but by comparison with other fruits one is justified in selecting the value for CO_2 evolution after the rate became constant. However, irrespective of the actual magnitude of the anaerobic CO_2, the respiration rate rises sharply in the low oxygen tensions until about 5 per cent O_2. Above that concentration the slope of the curve decreases until a value of approximately 35 per cent O_2 is reached, above which there is no change. Actually the difference between air and the higher oxygen tensions is hardly significant. While the curve was selected for the maximum respiration rates, a similar relation was obtained for the preclimacteric minima whenever they could be detected. In the high oxygen atmospheres the curves rise almost immediately after the commencement of differential treatment. A continuous recording of CO_2 production might facilitate detection of the minima even where the changes are rapid. Another possibility would be to conduct the experiment at a somewhat lower temperature, but in this case the measurements for the low oxygen tensions may be too small to show significant differences. However, in comparing the respiration-oxygen tension curve with the curves for respiration-time at different oxygen concentrations (fig. 2, 3), it is readily seen that the former represents the trends reasonably well, not only for the climacteric peak but throughout the course of respiration.

Effect of oxygen concentration on the respiratory quotient at 15°C.—In the experiments described thus far, the carbon dioxide evolution was used as an index of the respiratory activity of the fruit. The question arises as to what extent this index represents the aerobic process, and whether a certain percentage of the CO_2 measured may be accounted for by reactions other than complete oxidation. A definite answer to this question can be obtained by parallel determinations of the gas exchange and the chemical analyses of the end products formed. However, a suggestion as to the nature of the respiratory process may be gained by simultaneous measurements of CO_2 evolution

and O_2 absorption. For this purpose the procedure worked out by Haller and Rose (1932) was adopted with some modifications (Masure, 1938; Hansen, 1942). The respiration jar was provided with a 200 ml funnel inserted through a hole in the bottom. This funnel was filled with 100 ml of 2 N KOH through one of the glass tubes in the glass top of the jar. Then all the open ends were closed with the exception of one of the tubes, which was connected to a 500 or 1000 ml graduated cylinder of oxygen under constant hydrostatic pressure. At the end of the measurement period, the volume of oxygen displaced was recorded, and the funnel of alkali was emptied into a 500 ml volumetric flask and rinsed twice with 170 ml H_2O. After making up to volume, 15 ml aliquots were pipetted into hydrometer cylinders, to which 200 ml H_2O and 10 ml of 1.5 N $BaCl_2$ were added and titrated with 0.15 N HCl. This method of measuring CO_2 evolution in a closed system (referred to as method II) was compared with the previously described method I, in which a continuous stream of air was passed over the fruit and through the absorbing solution. The comparison made for air, 10, 5, and 2.5 per cent O_2 showed close agreement with deviations of the order of 2 per cent.

This simultaneous measurement of CO_2 evolution and O_2 absorption was used in several tests, of which experiment 6 can be cited as representative. In this experiment (fig. 5) the fruit was picked on June 16, 1943, and placed for one day in air at 350 ml/min. Differential treatment started on June 17 after the first CO_2 determination. While oxygen absorption was measured, the air and gas mixtures were discontinued and the jars connected to the oxygen graduates. Length of test varied from one and one half to four hours, depending on the intensity of respiration. Figure 5 shows that the nature of the curve for O_2 absorption did not differ materially from that for CO_2 evolution. The minimum and climacteric values as well as the slopes of the curves were approximately the same. The actual magnitudes were plotted in ml instead of mg of CO_2 so that comparisons might be drawn more readily. The O_2 rates were consistently higher than the CO_2 rates, resulting in a respiratory quotient (R.Q.) of less than one. In the case of the fruit in air there was a slight tendency for the R.Q. to increase until June 21, that is, until two days before the climacteric peak when the maximum R.Q. of .948 was reached. The subsequent course is that of a gradual decrease. A similar pre-climacteric condition was observed for the 10 per cent O_2 treatment. With the 5 per cent O_2 there was a decrease from .922 on June 17 to .806 on June 22, followed by an increase to .960 on June 29 after the climacteric peak. In 2.5 per cent O_2 there was a consistent rise from .784 on June 19 to .985 on July 1. Generally, there appeared to be somewhat higher R.Q. values in the lower oxygen concentration, but the differences were not large enough to suggest basic deviations from the normal respiratory process.

Total carbon dioxide production as affected by oxygen concentration.—In all the experiments on avocado fruit respiration it has been observed that under aerobic conditions the climacteric rise is a constant feature. The slope of the curve and the magnitude of the maximum can be altered by external factors, but they cannot be eliminated. It is intriguing to conjecture as to what it is that induces the sudden rise and what determines the time for the peak. If the highest rate of respiration coincides with the disappearance of certain substrates, then all the fruit samples in one lot may be expected to produce the same quantities of the end product irrespective of treatment. Attempts were therefore made to find out what are the total amounts of CO_2 formed between the first reading and the climacteric peak. The figures obtained will be referred to as cumulative CO_2 values. In experiment 3 (fig. 6) the hourly rates shown in table 4 were multiplied by 24 and each value added to the previous one, so that the final points on each curve give the total amount of CO_2 evolved up to that date. For those days in which no respiration measurements were available, interpolated rates were found on the respective graphs. The average CO_2 accumulated for 2.5, 5, and 10 per cent O_2, and for air was 14.10 grams per kg of fruit with a deviation of 11 per cent from the mean. This may appear to be high variability, but it should be remembered that the respiration of the last day, when the climacteric peak occurred, was at a maximum and could contribute greatly to the error. This was particularly true in the case of the control, because of the short time interval between the initial reading and the climacteric maximum. If the peak occurred early in the day and the CO_2 was calculated for the entire 24 hour period, the error could well be considerable. The fruit in the air jar, for example, produced on the day of the peak (April 27) 3.03 grams CO_2, which was 19.3 per cent of the total accumulated up to that day. This source of error doubtless could be eliminated by a continuous recording of the respiration results.

The cumulative CO_2 values for the experiment just described were compared with those from several other tests and are presented in table 6.

In experiment 8 the cumulative CO_2 for several additional oxygen concentrations was calculated and found to be 14.39, 15.98, and 17.55 for 15, 50, and 100 per cent O_2. Here again the deviations from the mean are most conspicuous for the higher oxygen tensions, and the explanation for this behavior may be the same as presented above in connection with the discussion of figure 6. There is an indication in this table that the cumulative CO_2 from initial to climacteric peak decreased as the season advanced. This is particularly true for the 1944 season, because an early sample of ex-

TABLE 6. *Cumulative CO_2 values in grams per kilogram of fruit.*

Exp. number	Date of initial reading	Per cent oxygen 2.5	5.0	10.0	Air	Average
3	4–20–43	14.10	13.12	13.54	15.65	14.10
5	5–26–43	11.95	....	....	13.78	12.87
6	6–17–43	11.89	12.70	11.33	13.87	12.45
7	1–12–44	19.19	23.36	20.62	23.77	21.74
8	7– 5–44	13.88	14.64	15.56	15.03	14.79

periment 7 could be compared with the late sample of experiment 8. Whether this difference can be accounted for by the depletion of some respiratory substrate cannot be determined at the present time. It is known that the sugar content of avocados decreases with the advance of the season, but it will be pointed out in the discussion that free sugars cannot account for the total CO_2 produced.

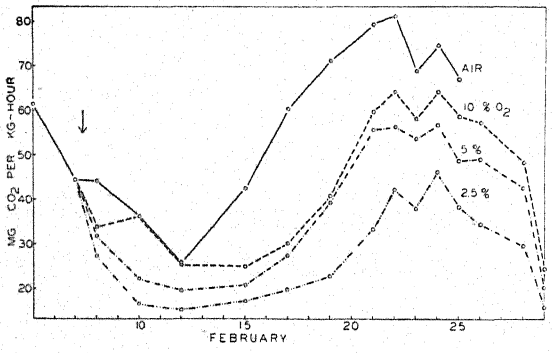
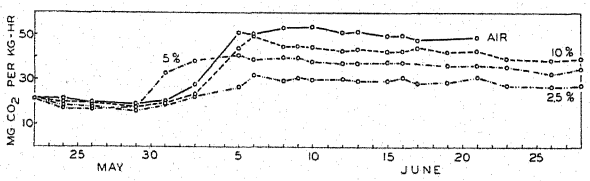


Fig. 7. Effect of oxygen tension on respiration of Fuerte avocados at 10°C. (Arrow indicates beginning of differential treatment.)

Oxygen tension effects in relation to temperature.—The results of experiments at 15°C., as described above, were compared with observations on the effects of oxygen on avocado respiration at 10°, 7.5°, and 5°C. Two or more tests were conducted at each of these temperatures. In experiment 9 the fruit was kept at 10°C. until it passed the climacteric stage and became edible, while in experiment 10 the fruit was placed under air at 15°C. after two weeks of storage in different oxygen concentrations at 10°C. The avocados for experiment 9 were placed in air on February 3, 1944, and differential treatment started on February 7, following the respiration measurements. The results in figure 7 indicate an initial value as high as in most experiments at 15°C. The rate of decrease from the initial is very marked with a slope steeper than that for the climacteric rise. The time interval between the initial and preclimacteric minimum was from eight to eleven days, while at 15°C. it varied from two to four days. The period from picking to the peak in respiration in the case of air and 10 per cent O_2 was twice as long at 10°C. as at 15°C. However, the differences between treatments were slight as far as the time required to reach the peak was concerned. At the minimum stage the actual respiration magnitudes were significantly different from the control for 2.5 and 5 per cent O_2, but not for 10 per cent O_2. The relatives rates of CO_2 production at the peak were 100, 79, 70, and 55 for the progressively decreasing oxygen concentrations. Reduced oxygen also had a marked influence on the softening process. On February 25 (after 22 days) most of the fruit in the control jar was softening, with some in the soft edible stage. On March 4 (after 30 days of storage) the avocados in jar 18 (2.5 per cent O_2) were found to be only slightly soft. Fruit quality was good in all cases.

The effects of change of temperature coupled with transfer to air were observed in experiment 10. Since the avocados for this experiment were picked six weeks later than for experiment 9, the onset of the climacteric rise took place after eight days in air, 10, and 5 per cent O_2. The rise in 2.5 per cent O_2 was very slight during the two weeks at 10°C. Hence the three highest O_2 concentrations exhibited a generally decreasing rate of CO_2 evolution when transferred to air at 15°C., while the fruit in the 2.5 per cent O_2 showed the typical rise followed by a drop. When the experiment was com-



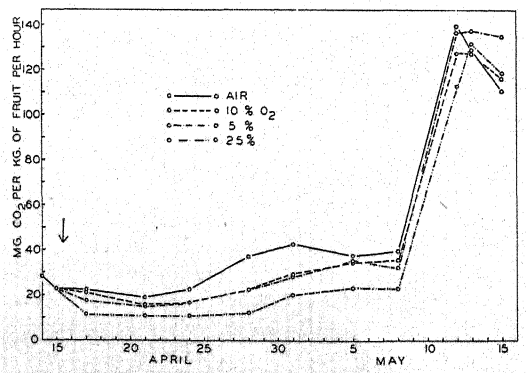


Fig. 8 (above). Effect of oxygen tension on respiration of Fuerte avocados at 7.5°C.—Fig. 9 (below). Effect of oxygen tension on respiration of Fuerte avocados at 7.5°C. (Arrow indicates beginning of differential treatment. On May 11 all jars transferred to air at 15°C.)

TABLE 7. *Respiration in air at 15°C. after storage in different O_2 at 5°C. (a = 49 days at 5°C., b = 25 days at 5°C.).*

Condition of storage % O_2	Days after transfer from 5°C. First a	First b	Second a	Second b	Third a	Third b
2.5	129.3	105.8	147.4	121.7	147.6	130.7
5.0	154.7	116.6	166.1	133.3	130.1	139.8
10.0	148.8	114.3	149.7	126.8	140.7	125.3
air	131.3	111.4	136.7	115.2	120.6	127.6
	Fourth b	Fifth a	Sixth a	Sixth b	Seventh b	
2.5	136.6	136.1	122.1	116.5	112.5	....
5.0	145.9	....	110.1	111.3	104.9	....
10.0	134.6	129.8	108.8	110.6	102.4	....
air	138.3	112.9	89.4	115.9	107.4	....

pleted on April 4, the fruit in the containers with the two lowest oxygen concentrations were in edible state and of much better appearance than the control, in which brownish discoloration of the skin was recorded.

Since marked differences were observed between the respiratory behavior of avocados at 5°C. and at 10°C., it became advisable to study oxygen effects at narrow intervals of temperature. Three experiments were conducted at 7.5°C. In experiments 11 and 12 the fruit was transferred to 15°C. after 26 and 24 days of storage, respectively, while in experiment 13 softening took place at 7.5°C. The avocados for the last test were subjected to differential treatment on May 22. The trends in respiration are illustrated in figure 8, showing a somewhat different picture than in previous experiments. The rise in CO_2 evolution started one day earlier in the 5 per cent O_2 jar than in the other treatments. This anomalous behavior may be explained by the presence of one or more avocados that were considerably more advanced in their softening process. Since there is no sharp peak in the respiration curves at this temperature, comparisons can be made only for the different levels of CO_2 production. The relative maximum rates were found to be 100, 84, 72 and 60 for air, 10, 5, and 2.5 per cent O_2 respectively; the differences recorded for the minima were hardly significant. The softening process was prolonged markedly as compared with previously described tests at 15°C. and 10°C. There was a time lapse of a week to 10 days in softening between the treated samples and the control. After 41 days almost all the avocados in 2.5 per cent O_2 were edible and of better quality than the fruit subjected to air. However, the differences between the several oxygen concentrations were less marked.

In two other experiments the fruit was transferred from 7.5°C. to air at 15°C. In experiment 12 (fig. 9) this happened on May 11, 25 days after picking. The response of the fruit to this change differed with treatment. In the control the rate of CO_2 evolution was on a definite decline; in the 5 and 10 per cent O_2 there was little difference in rate between May 12 and May 13, followed by a decided decrease. On the other hand, the fruit subjected to 2.5 per cent O_2 went through the climacteric cycle. These trends of the several jars can be traced to their behavior at 7.5°C. prior to the transfer. The control was the only fruit which appeared to have a somewhat more defined peak in respiration on May 1, while in the remainder the increase was gradual without any typical climacteric. When the experiment was discontinued on May 16 (after 33 days) the fruit subjected to 5 and 10 per cent O_2 was in best condition, uniformly soft and edible, with excellent flavor, color, and texture. There was slight browning in the 2.5 per cent O_2 treatment, while the control avocados did not soften as uniformly as the rest and showed more of the brownish discoloration.

From the tests at 10°C. and 7.5°C. it seems evident that the differences between the various oxygen concentrations are not so pronounced as at 15°C. This behavior was borne out even more strikingly in the studies at 5°C. In experiment 14 the fruit was picked on April 6, 1943, subjected to different oxygen concentrations on April 10, and left for 49 days under this treatment at 5°C. On May 26 all the jars were transferred to air at 15°C. In experiment 15 the fruit was received on May 26, 1943, and placed in air at 5°C. for two days. Differential treatment lasted from May 28 to June 22, that is, for 25 days, after which time all jars were transferred to air at 15°C. The rate of CO_2 evolution was of the order of 10 to 20 mg per kg-hour, showing little or no response to oxygen tension. When the fruit was placed under air at 15°C., there was a considerable difference in the course of respiration for these two experiments, as shown in table 7.

In experiment 15 (designated by "b" in the table) all the avocados irrespective of treatment showed upon transfer an increasing rate of respiration, while in experiment 14, with twice as long a storage period, the 2.5 and 5 per cent O_2 fruit behaved similarly. The fruit subjected to 10 per cent O_2 and air had a declining rate of CO_2 evolution, indicating a post-climacteric behavior. They apparently had undergone physiological changes at 5°C. that are characteristic of the climacteric phase, even

though this could not be detected by the respiration measurements. Pratt and Biale (1944) have shown by the triple response of pea seedlings that such changes may be observed at 5°C. The condition of the fruit in experiments 14 and 15 at the end of the softening period was not satisfactory. A large percentage of the avocados did not soften properly, with discoloration occurring both on the surface as well as inside the flesh. Treatment with decreased oxygen had not materially improved the condition of the fruit when subjected to 5°C.

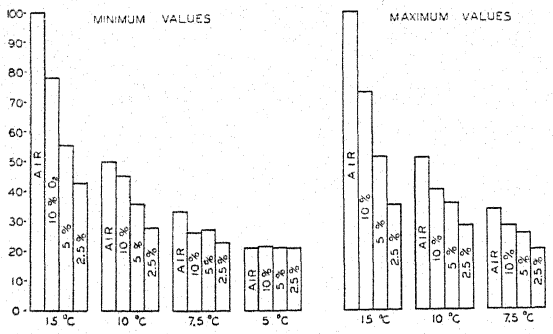


Fig. 10. Relative respiration rates of Fuerte avocados under different oxygen pressures in relation to temperature.

By way of summary, figure 10 is included to show a diagrammatic comparison of the effects of oxygen for the several temperatures. The lowest and the highest respiration values were selected. The preclimacteric minimum and the climacteric maximum magnitudes for air at 15°C. were taken as 100 and all other values computed with reference to those rates. Since no definite increase took place at 5°C., only minimum rates are given for that condition. This diagram illustrates clearly that with a decrease in temperature, oxygen tensions of the range used cease to be the determining factors for the rate of CO_2 evolution of Fuerte avocados.

Discussion of results.—The results of the investigations presented in this paper point to a certain relationship between the effects of oxygen and temperature on the respiration rates of Fuerte avocados. At low temperatures the effects of oxygen concentrations are minimized with the consequence that the temperature coefficient (Q_{10}) is markedly different for the several oxygen levels. Thus at 2.5 per cent O_2 the Q_{10} for the minimum respiratory rates in the range of 5°C. to 15°C. has a value of two, while the corresponding Q_{10} magnitude in air is two and one half times as high. The temperature coefficient in this range for the peak respiration rates cannot be determined with precision because of the uncertainty in establishing the exact date of the climacteric maximum at 5°C. However, an approximation can be obtained by following the effects of avocado emanation on pea seedlings along with the CO_2 measurements. As indicated above the maximum response of the etiolated seedlings coincides with the climacteric rise and takes place at 5°C. as well as at higher temperatures. If the respiration rates corresponding to the maximum pea response are selected, we find the Q_{10} in the range of 5°C. to 15°C. for the peak values to vary from approximately four in 2.5 per cent oxygen to about eight in air.

From the standpoint of fruit storage this interrelation between temperature and oxygen tension effects suggests the advisability of using temperatures higher than 5°C. for storing Fuerte avocados in subnormal oxygen atmospheres. The use of fairly high temperatures is often correlated with better fruit appearance after removal from storage. It may also mean considerable reduction in chilling injuries to sensitive varieties. Wardlaw and Leonard (1935) reported marked chilling effects for some West Indian avocados when exposed for fifteen days to 53°F. (11.7°C.). These effects are frequently associated with browning of the skin and discoloration of the flesh. They ascribed the skin symptoms in certain varieties to subnormal oxygen, while the condition of the flesh was thought to be due either to high CO_2 or to a combination of high CO_2 and low O_2. These physiological reactions were found to vary for different varieties ranging from high resistance to complete sensitivity to subnormal oxygen or modified air storage. In the case of the Fuerte variety as grown in Southern California the available results appear to show that chilling injuries are limited to temperatures below 7.5°C.

These low temperature effects introduce complications in determining the storage life of avocados. In this work we have used the course of respiration as a criterion for the condition of the fruit. Softening followed the peak in CO_2 production in air as well as in the different oxygen atmospheres employed. Wardlaw and Leonard (1935) claimed that the climacteric rise is not correlated with the color change in coloring varieties or with softening of the flesh. They based this conclusion on the fact that the above manifestations occurred after the peak in respiration was attained. We know that at least softening in the non-coloring Fuerte variety is always a function of the incidence of the climacteric. The fact that complete softening occurs after the respiration maximum does not preclude the possibility of an earlier initiation of the process. As far as storage behavior is concerned, the relevant point is that delayed climacteric rise means delayed maturation, and that this condition can be brought about by reduced oxygen.

In addition to respiration rates some workers have used total or cumulative CO_2 as a criterion for storage life. Kidd and West (1930) calculated the total amount of carbon dioxide produced by apples between picking and death by fungal attack. They found this quantity to be reasonably constant for several fruit samples stored at three different temperatures. When converted to equivalent sugars oxidized, they observed that only 16 to 20 per cent of the reserve carbohydrates were used up. Similarly, Smock (1942) investigated the total amount of

CO_2 evolved by McIntosh apples in ordinary cold storage as compared to controlled atmosphere storage. The cumulative CO_2 values between picking and the end of storage life (based on marketability) were approximately constant under the two treatments, but they varied somewhat from one season to the next.

In the case of the Fuerte avocado we found a condition similar to that for apples, but insufficient data are available to recommend the cumulative CO_2 values rather than the rate of respiration as a storage criterion. By using the climacteric peak as the index of storage termination a more objective evaluation was employed. One of the reasons for calculating cumulative CO_2 was to ascertain whether sugars could account for the CO_2 evolved. If the value of 21.74 grams of CO_2 is selected from table 7 for a sample of avocados picked in January, and if the assumption be made that the process involved is that of complete oxidation, the sugars consumed would amount to 14.8 grams or approximately 1.6 per cent of the fresh weight of the flesh. This is a much higher sugar content than that reported by Church and Chace (1921) for January fruit. Moreover, they found that sugars were not depleted during storage. There is also some evidence available that fats could not account for the respirable substrates either. It appears that more complete biochemical data are needed to explain the physiological changes described in this paper.

SUMMARY

The effects of oxygen on CO_2 evolution by Fuerte avocados were studied in the range of 0 to 99.4 per cent O_2 at 15°C. At 10°, 7.5°, and 5°C. the response to 2.5, 5.0 and 10.0 per cent oxygen was compared with the behavior in air. Apparatus for preparing the gas mixtures is described, as is the effect of different rates of flow.

When the oxygen concentration is reduced to values below that of air, the rate of CO_2 evolution is markedly affected, with the climacteric peak delayed and suppressed in magnitude. At oxygen tensions higher than air no significant differences occurred as compared with the controls. Under anaerobic conditions the rate of CO_2 production is markedly inhibited with the climacteric rise completely lacking. When the oxygen concentration affected the respiratory course, there was also a pronounced influence on fruit softening. At 2.5 per cent O_2 it took approximately twice as long for the fruit to soften as in air.

The rate of oxygen absorption by the fruit was studied at 15°C. under 2.5, 5.0, and 10.0 per cent O_2 and in air. It was observed to follow closely the rate of CO_2 production with some tendency for a higher respiratory quotient at low oxygen tension.

The total amount of CO_2 given off by the fruit during the period ending with the climacteric peak was found to be approximately constant for the several modified atmospheres at 15°C.

The effects of oxygen tension on respiration were markedly modified by temperature. Differences in CO_2 evolution of the fruit due to the several treatments were most pronounced at 15°C. and least at 5°C.; at 10°C. and 7.5°C. the respiration rates of the avocados were less affected by oxygen than at 15°C. but considerably more than at 5°C. Fruit softening was found to be closely related to the respiration trends.

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ON FUNGAL DAMAGE TO SUN-EXPOSED COTTON DUCK [1]

Robert K. Zuck and William W. Diehl

OBSERVATIONS ON the kinds of fungi found on cotton fibers and cotton cloth are numerous. In many instances, the investigators have been primarily concerned with fungi of the more superficial types growing on the sizing and other non-cellulosic carbon compounds of the fabric. The reports, however, of the fungi responsible for the decomposition of fabric such as awnings and tentage under actual conditions of outdoor exposure when not in contact with the soil, are much more limited (Broughton-Alcock, 1919; Thaysen and Bunker, 1927) and have not been quantitatively evaluated. Techniques employed for isolating organisms supposed to be primarily responsible for the actual decomposition of cellulose in cotton fabric have frequently erred on two major counts. Those methods employing nutrient agars with sugars as a source of carbon favor the rapid growth of any fungus or bacterium suited to that substratum, whether or not it be a cellulose decomposer. The method using filter paper and mineral salts agar while isolating some cellulose decomposers fails to detect many others because it does not take into account the fact that cotton fibers in gray duck supply vitamins B_1, B_6, and biotin (Robbins and Ma, 1942), as well as other possible nutritional constituents for fungi having special requirements (Robbins and Schmitt, 1945). Gray duck in contrast to bleached duck retains the protoplasmic contents left in the lumina of the natural fibers, as well as fragments of seedcoats, leaves, and the like. Bleaching and scouring largely remove these remnants. The natural cotton fiber, on a moisture-free basis, contains the following percentages of elements expressed as means of several readings (Beeson, 1941): 0.59 per cent K, 0.13 per cent Ca, 0.09 per cent Mg, 0.054 per cent P, 0.05 per cent S. Raw cotton on a moisture-free basis is reported (Marsh and Wood, 1942) to contain 0.2 per cent N, and bleached cotton none. Of the data available on the minor elements, iron and manganese are both present on a moisture-free basis in raw cotton as 190 parts per million. It is presumed, however, that other minor elements are present.

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This paper reports the presence in and effects on gray duck of certain species of fungi belonging to genera heretofore unappreciated as decomposers of cotton fabrics under conditions of unshaded aerial exposure, the significance of their developmental sequence and the extent of their damage to the fabric as determined quantitatively by a device herein described.

MATERIALS AND METHODS.—Fabrics exposed for different periods at Miami, Fla., New Orleans, La., Baltimore, Md., Washington, D. C., and in Panama have been made available for examination. Most of the observations and illustrations here recorded were made from the samples from Florida owing to the larger number of Florida samples representing exposures of varying periods of time. Examination of the fabrics was made macroscopically by both reflected and transmitted light using a 60-watt Mazda lamp as the illuminant, and microscopically by reflected light with the stereoscopic dissecting microscope for detection of fungal structures. Stainless steel jeweler's tweezers with finely ground points were used for picking off individual fruiting structures. Each fabric sample was assigned a number and each fungus fruiting area examined was outlined in ink and lettered for ease of reference.

Isolation cultures were from spores from these structures by streaking on cotton extract agar plates (Zuck, 1946). Reinoculation with these isolates was then made on sterile strips of gray duck, $4'' \times 1/2''$, in test tubes, the tubes being placed in a saturated atmosphere at room temperatures. One-quart mason jars were used for saturated atmosphere chambers. The ability of a particular fungus to grow on the fabric without additional nutrients was thus demonstrated.

The fabrics, unless otherwise noted, were gray duck (Hooper's lot K-99 No. 6—44″ width, 25.34 oz. per linear yd.). Exposures had been made at 45° facing south on wooden racks, with free circulation of air on both surfaces except for tentage and other items mentioned separately later.

It was evident that different areas on a fabric, particularly those points where fungal growth was well developed, were weaker than others—an obvious condition to which, nevertheless, little attention has been given. It seemed highly desirable to obtain some quantitative expression of these differences; but no suitable instrument was available and a survey of the various types of burst-testing machines in standard use for fabrics and paper disclosed that none met this requirement. An apparatus was therefore devised by adapting the pressure tester used by Culpepper and Magoon (1924) in their study of resistances of plant tissues to puncture. This was accomplished as follows: A clamp was made from two pieces of $3/16''$ boiler plate $9\frac{1}{2}'' \times 6''$ with a $5/8''$ square hole in the cen-

ter of each plate, the plates faced with thin cardboard to provide friction to hold the cloth sample firmly when the plates were clamped together with thumb-screws. The area of cloth being tested was at a selected point showing through the holes in the plates. The testing apparatus, consisting of a rod welded to a long spring inside a glass tube with a scale graduated in 0.2 kg. up to 14 kg., was adapted to the needs at hand by threading on the free end of the rod a fitting 1.5 mm. in diameter, i.e., covering an area of 1.76 sq. mm. This size fitting by experience proved most useful for the weights of fabrics tested. In this gray duck two yarns in the warp and filling directions each were covered by this fitting. The test measured primarily the strength of the four yarns and secondarily the tightness of the weave. Each test was made by holding the glass cylinder in both hands and applying force until the pressure burst the fabric, the reading being taken by a second person at that instant.[2]

OBSERVATIONS.—*Examination by reflected light.*—Gray duck as it appears newly woven is characterized (fig. 1) by many spots or flecks of darker color than the main body of the fabric. These darker flecks due to the local presence of substances foreign to the lint when spun into the yarn remain after it is woven as a characteristic feature of this type of fabric. After one year's exposure in Florida the lower surface, i.e., the shaded surface, of this fabric (fig. 2) had assumed an entirely different appearance, being distinguished by numerous "mildew spots" or dark-colored areas which were individual or contiguous fungal colonies. The upper surface (fig. 3) by contrast was relatively free of these dark areas. Samples of gray duck subjected in Florida to exposures of 3, 6, 9, and 12 months showed on their lower surfaces (fig. 4, 5, 6, and 2, respectively) progressive increase in the sizes of these dark spots as well as a progressive increase in the total area covered by them. A sample of gray duck, vertical hang, after exposure for but fifteen days in August, 1945, at Beltsville, Md., was velvety on both sides with an appressed mould growth which was almost exclusively *Alternaria* sp.; this growth tended to disappear within the next two months leaving a condition and appearance similar to that of the Florida specimen exposed for three months (fig. 4). Even after 18 months' Florida exposure, however, certain light-colored or uncolored areas with little or no fungal growth were still evident, but relatively very few. In all these samples the upper surface, i.e., the surface exposed toward the sun, was practically unspotted when viewed by reflected light. A sample of gray duck, supposedly 10-oz., exposed for one year in Louisiana (fig. 9) is similar in gross appearance to the one exposed for the same length of time in Florida.

A sample of 8-oz. mineral khaki (iron and chromium hydroxide precipitated in the fibers) after exposure for one year in Florida showed fewer and smaller fungal colonies (mildew spots) than did the untreated gray duck. Interestingly, however, this khaki, being thinner than the other ducks here referred to, bore fungal colonies evident not only on the lower surface (fig. 7), but on the upper surface (fig. 8) also, each colony from the lower surface tending to penetrate to the upper surface of the fabric.

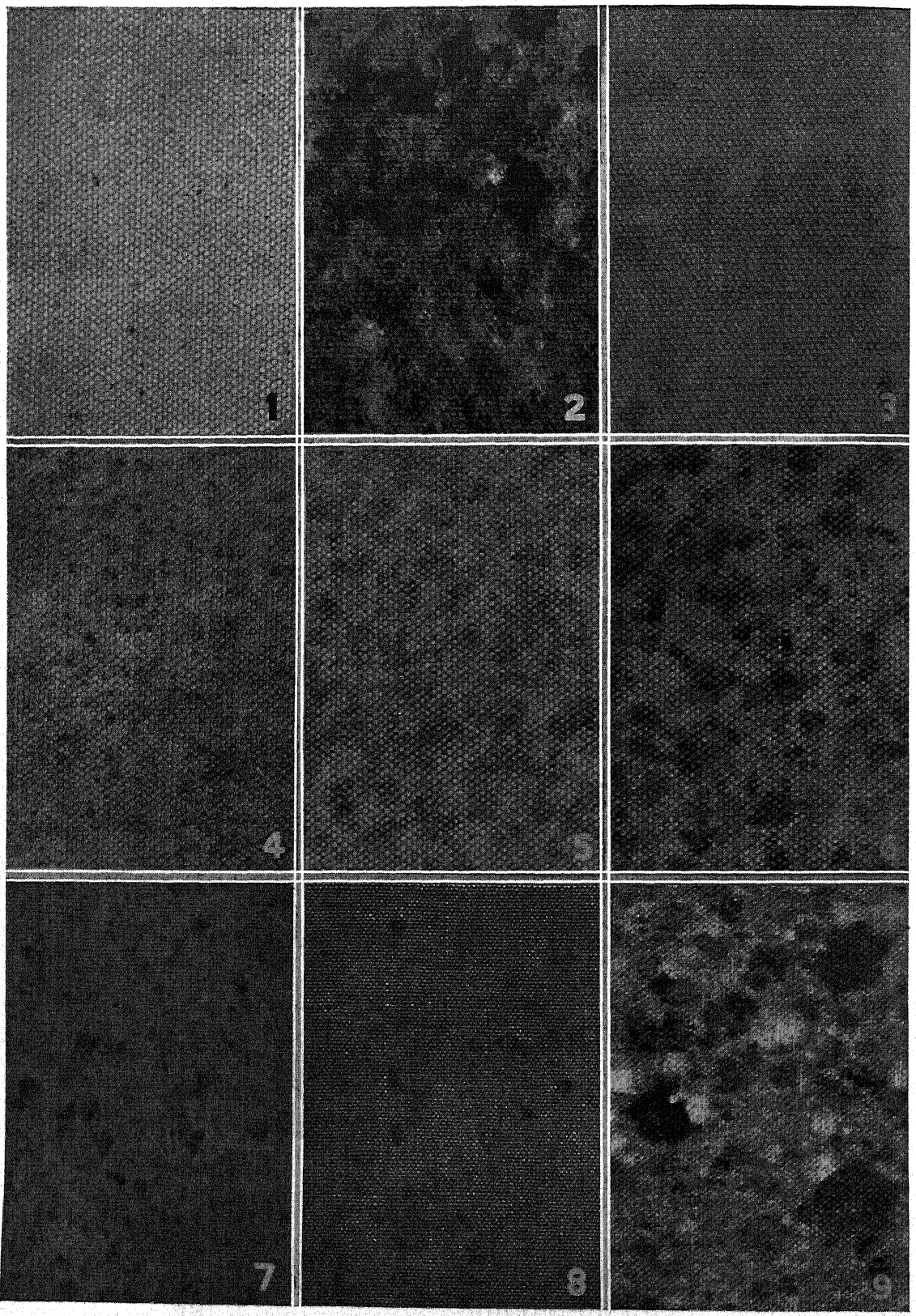
In each of the samples here cited the incidence of fungal colonies was strikingly uniform, the illustrations (fig. 1 to 9) being representative. It is noteworthy that the incidence of attack as indicated by the growth on the lower surface was practically the same on the sample exposed for but three months (fig. 4) as on those exposed for a year or more. Numerous fungal fructifications evident by means of a hand lens (fig. 13) are discussed below relative to their identification.

Examination by transmitted light.—When the fabrics cited above were viewed by transmitted light, the discoloration due to the fungal colonies was much more strikingly evident than when viewed by reflected light. In the sample with but 3 months' exposure in Florida the contrast between a view of the lower surface by transmitted light (fig. 10) and by reflected light (fig. 4) was notable, the darkened individual warp and filling threads showing fungal attack progressing in all four directions. The colonies superficially evident on the lower surfaces extended deeply into the fabric—in the gray duck not appearing on the upper surface. In the thinner 8-oz. khaki material noted above, however, the colonies showed on both surfaces.

Comparative tests of fabric strength in different areas after aerial exposure.—The data secured by means of the modified Culpepper and Magoon apparatus are summarized in figure 18. These show that after twelve months' Florida exposure the bursting strengths of those areas of gray duck containing fungal colonies have decreased from an original bursting strength of 11 kg. to 2.9 kg. per 1.76 sq. mm. (i.e., 6.25 kg. to 1.6 kg. per 1.0 sq. mm.). In contrast, however, those areas relatively free of fungal attack as evidenced by their light color showed a slight but inconsistent drift downward from the original bursting strength. In the case of the 18-month Florida exposure, utilizing, however, a lighter-weight duck (Hooper's lot H P 81 No. 8—36″ width, 18 oz. per linear yd.), the lighter colored areas likewise retained their resistance to puncture. The mineral khaki samples of both 6 months' and 12 months' exposure in Florida showed small differences in strength between fungal spots and their absence on the same piece of fabric, but considerably greater loss in strength after twelve than after six months. The fabric with 2¾ years' exposure in a vertical hang in Baltimore also showed little difference in bursting strength between fungal and non-fungal areas.

Microscopic examination of fibers.—Microscopic examination of fibers removed from the darker areas

[2] A new device utilizing these same principles is being perfected by the writers in order to simplify the operation and improve its efficiency.


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showed them to contain dematioid hyphae (fig. 14), explaining thereby the dark color of the spots whence they came. Fibers from the light areas were more rarely found to contain hyphae. When these fibers from the black spots were treated by the well-known sodium hydroxide-carbon disulfide swelling test (Thaysen and Bunker, 1927), they failed to swell (fig. 15) in the beading pattern typical of normal fibers (fig. 16). When, however, fibers from the clearer areas of the samples exposed in Florida for 3, 6, 9, 12, and 18 months were so treated, they showed a beaded condition (fig. 17) somewhat similar to that of undamaged fibers.

Fabrics exposed in cities.—The sample of 10-oz. gray duck after exposure, vertical hang, for 2¾ years in Baltimore, Md. (fig. 11), was of a generally dirty gray color on both surfaces with some spotting as viewed by reflected light. When examined by transmitted light, however, there were discernible within the fabric many dark areas usually less than 6 mm. in diameter (fig. 12). Critical search upon the surface and within the fabric failed to reveal any fungal fructifications with identifiable contents. Hyphae were not found within all of the cotton fibers from the dark areas and but rarely in the fibers from the less darkened areas. Many fibers, those from the dark areas particularly, revealed sculpturing suggesting an erosion pattern where chemical action, hyphae, bacteria, etc., may at some time formerly have been present. On the surface of many fibers were cells of *Protococcus viridis* Ag., while bacteria in and on fibers were evident in stained preparations.

An untreated boat cover that had been in constant service on the Washington, D. C., waterfront for several years afforded an interesting comparison with the sample from Baltimore. This boat cover had become uniformly very much tendered, breaking with very little pressure, and, although of a slightly less dirty-gray color than the fabric exposed in Baltimore, lacked the dark areas so noticeable in that specimen except in folds near and at the ground. It seemed noteworthy that fungal hyphae were practically non-existent in the fibers from this fabric although sculpturing as noted above, algal cells, and bacteria were found upon them.

Identity of the fungi present on gray duck after aerial exposure.—After exposures of six months or more in Florida and in Louisiana, none of the samples of gray duck bore any indication of the hyphomycetous fungi usually evident after shorter exposures, but numerous minute fructifications were evident with a hand lens on the darkened areas (mildew spots) and more rarely on the light-colored areas. Examination by means of the stereoscopic dissecting microscope disclosed that many of these were superficial, others embedded or erumpent from within the fabric (fig. 13). When on the surface, these fructifications could be removed intact by means of a knifepoint for microscopic examination, and when embedded in the fabric, they could be picked out by the especially sharpened tweezers described above. These fructifications were all angiocarps in various stages of maturity referable to but two genera of the Ascomycetes and to but four form-genera of the Fungi Imperfecti.

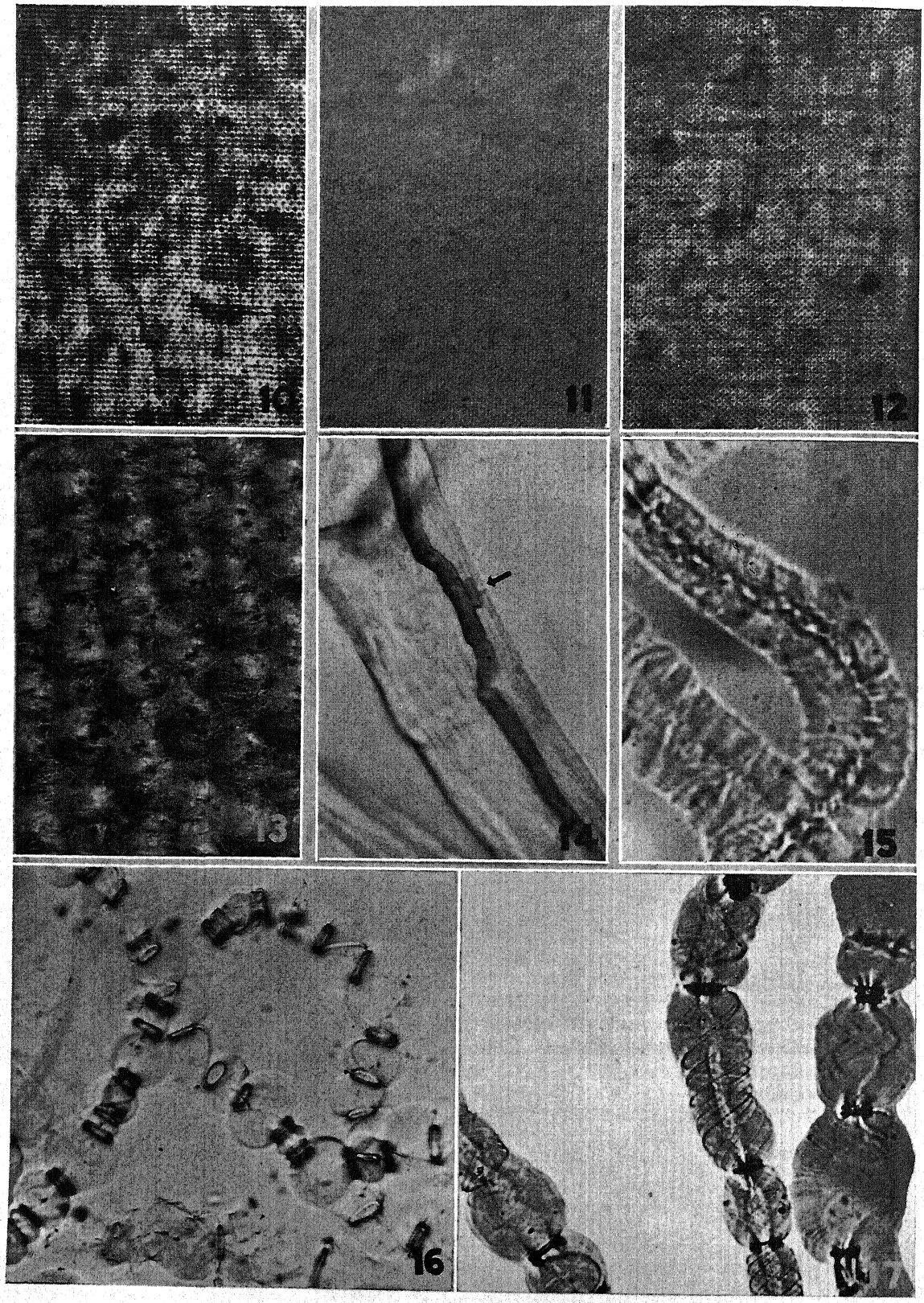
Most of these angiocarps, however, were either empty or too immature for adequate specific identification, and ordinary moist-chamber attempts to add to the components of this flora were unproductive.

Those bearing asci were recognized as belonging to two species of *Leptosphaeria,* here noted as "*L.* sp. no. 1" and "*L.* sp. no. 2," and to one species of *Ophiobolus.* None of these seemed to be identical with any species now well-defined and recognized in the literature, although it is realized that a critical study of the numerous recorded species of *Leptosphaeria* and of *Ophiobolus* might be expected to disclose identities where descriptions alone are inadequate. The perithecia of the two species of *Leptosphaeria* were relatively frequent whereas but two perithecia of the *Ophiobolus* were encountered in recognizable condition.

Pycnidia were more numerous than perithecia but were usually indistinguishable from them except when preparations were examined under the compound microscope. As in the case of the perithecia above referred to, most of the pycnidia examined were found to have evacuated their contents or to be immature. Of the matured pycnidia five different kinds were distinguishable and are reported as follows: *Hendersonia sarmentorum* West., *Hendersonia* sp., *Phoma herbarum* West., *Diplodia* sp., and *Diplodiella Cowdellii* (Berk. & Br.) Sacc.

Fungi of the type reported above are found in nature characteristically as stem and leaf fungi. *Diplodiella Cowdellii* may properly be thought of as in the same category although it has been recorded hitherto (Grove, 1937) only from a cotton shower bath curtain in England. The paucity of species encountered in this study is in striking contrast to the great number of species, chiefly Hyphomycetes, reported from cotton when stored under humid or wet conditions or when in direct contact with the earth.

Fig. 1–9.—Fig. 1. Gray duck (Hooper's lot K-99 No. 6—44″ width, 25.34 oz. per linear yd.) as it appeared newly woven (× 1) (fig. 2 to 6 incl. represent samples (× 1) from the same piece of cloth after the several periods of aerial exposure in Florida at 45° facing south, showing discoloration (mildew) due to the presence of dark hyphae). —Fig. 2. Lower surface, after exposure for 12 months.—Fig. 3. Upper surface after exposure for 12 months.—Fig. 4. Lower surface after exposure for 3 months.—Fig. 5. Lower surface after exposure for 6 months.—Fig. 6. Lower surface after exposure for 9 months.—Fig. 7. Eight-oz. mineral khaki, lower surface after exposure for 12 months in Florida (× 1).—Fig. 8. Eight-oz. mineral khaki, upper surface from same sample as in figure 7, after 12 months' exposure in Florida (× 1).—Fig. 9. Ten-oz. gray duck lower surface after exposure of 12 months in Louisiana (× 1).


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Inoculations of strips of gray duck with the several isolates.—Isolates of four of the fungi enumerated above, *Leptosphaeria* sp. no. 1, *Leptosphaeria* sp. no. 2, *Hendersonia sarmentorum,* and *Diplodia* sp., were obtained in pure culture on cotton extract agar and transplanted to strips of sterile gray duck in test tubes kept in a saturated atmosphere. These were allowed to grow at room temperatures for two months. Abundant growth was noted as the hyphae proceeded from the site of inoculation at the upper end of each strip. When, at the end of two months, the fabric strips were removed from the test tubes and tested for breaking strength by pulling apart with the hands, these fabric strips were so weakened that they broke with great ease although the two isolates of *Leptosphaeria* caused the least weakening. After four months' growth, however, the isolate of *Leptosphaeria* no. 1 had tendered the fabric completely.

In the case of the *Leptosphaeria* isolates no perithecia were formed either on agar media or on the fabric in the test tubes. Pycnidial formation in the cultures of *Hendersonia sarmentorum* and *Diplodia* sp. was more abundant in the agar cultures than upon the fabric strips. These fungi did not lend themselves readily to isolation, owing to the fortuitous presence of large numbers of spores of the more rapidly growing species. In addition many perithecia contain only aborted or immature ascospores and the conidia of the pycnidial forms encountered have shown a low viability, or at least did not germinate well.

Discussion.—In all probability the role of the fungi herein considered as primarily involved in the decay of fabric during aerial exposure in sunlight, but without ground contact, has not been appreciated previously because these fungi have escaped detection by the methods commonly employed for their isolation. These fungi, owing to their slow growth under the normal conditions of diurnal and seasonal desiccation to which the substrata are exposed, fruit only after a lapse of several months, and, as the exposure period lengthens, more and more colonies produce angiocarps.

A direct correlation exists between the increased darkness of a colony or mildew spot as viewed by reflected light, the presence of angiocarps of the fungi, and the weakening of the fabric at that point. The dark color of the mildew spot, however, is due to the dark hyphae within the fibers, which weaken them, and the numerous dark angiocarps ultimately present tend to add but slightly to the darkening of the area. These fungi are well suited to withstand the extremes of drying, wetting, heating, cooling, and the intense rays of the sun; otherwise, they would not survive such conditions and be able to reproduce themselves. Thom and Phillips (1932) have pointed out that fungi with dark colored hyphae and spores are common upon the surfaces of decaying vegetation and that "these organisms predominate only in the presence of air and light." It is a natural supposition that similar fungi having brown hyphae are concerned with decay of fabrics. It is, however, to be expected that the slower-growing species found in fabric exposed to light and air but not in contact with the soil would compose a flora of different specific composition from that occurring upon fabrics long-exposed to direct contact with the earth. The species of *Phoma, Diplodia, Hendersonia, Diplodiella,* and *Leptosphaeria* here considered as significant in decay of gray duck are not revealed by the standard techniques usually employed in isolating fungi that appear on particular agar media following the planting thereon of fragments of fabric. These techniques applied to isolation from fabrics after exposure of three or four months

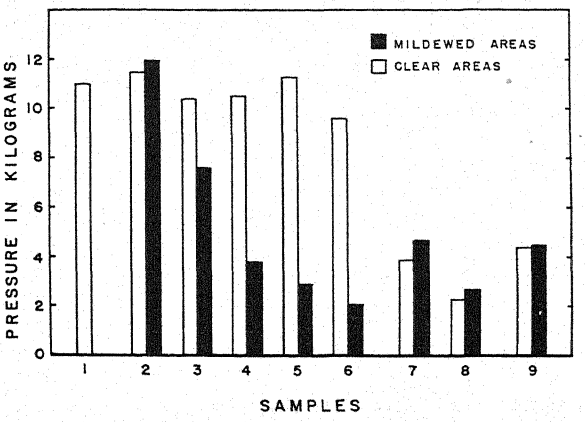


Fig. 18. Comparisons of fabric strengths after the several periods of aerial exposure including comparisons of the strength of mildew spots with that of clear areas on the same fabric sample as revealed by resistances to puncture (burst tests) expressed in kgms. per 1.76 sq. mm. Each column represents the mean of 5 tests of comparable areas on each sample. Samples nos. 1 to 6 refer to gray duck after exposures in Florida of 0, 3, 6, 9, 12, and 18 mos., respectively. Samples nos. 7 and 8 are 8-oz. mineral khaki after exposures in Florida of 6 and 12 mos., respectively. No. 9 refers to the sample of 10-oz. gray duck exposed in Baltimore for 2¾ years.

Fig. 10–17.—Fig. 10. From the same sample as figure 4, but viewed by transmitted light (× 1).—Fig. 11. Ten-oz. gray duck (12.51 oz. per sq. yd.), vertical hang, after exposure of 2¾ years in Baltimore, Md., showing discolorations (× 1).—Fig. 12. From same sample as figure 11 seen by transmitted light (× 1).—Fig. 13. Detail, lower surface, from the same sample as figure 2, showing erumpent pycnidia of *Hendersonia sarmentorum* (× 11).—Fig. 14. Part of a cotton fiber from the 18-month Florida-exposed duck showing dematioid hypha within it (× 1100). Note the hole through the wall of the fiber at point indicated by arrow.—Fig. 15. Fibers from discolored area (mildew spot) on 18-month exposed duck (same sample as in fig. 14) after treatment by NaOH + CS_2, showing the absence of beading (× 143).—Fig. 16. Fibers from unexposed gray duck (same sample as fig. 1) after treatment by NaOH + CS_2, showing beading (× 143).—Fig. 17. Fibers from clear areas of mildewed fabric (same sample as in fig. 14 and 15) after treatment by NaOH + CS_2, showing beading (× 143).

usually reveal only the presence of rapidly growing fungi, species of *Stemphylium, Alternaria, Penicillium, Aspergillus, Fusarium,* etc., while there is little likelihood that the slower-growing species here under consideration would be detected and evaluated. Even though some of these may be able under suitably moist conditions *in vitro* to decompose cellulose, it would seem that they are utterly unable to endure and function when exposed to recurrent short periods of drying and sunlight. On the other hand some species that can endure these conditions, for instance the *Alternaria* previously noted as having made considerable growth during the first 15 days' exposure, doubtless derive the major portion of their nutrition from materials in the yarn other than those fibers that lend it strength, since even after these pioneer fungi have been succeeded by others the resistance of the fabric to puncture remains unimpaired. This is no criticism of plating techniques employing either nutrient agars or mineral salts agar plus filter paper—they have their place especially in the detection of fungi causing deterioration of fabrics in contact with the ground or exposed for long periods, as in storage or shipment, to constantly humid conditions. Rather it is desired to point out that direct observation of fruiting structures *in situ* is requisite to give an adequate picture of the fungi involved in fabric decay under the conditions here considered. It should be significant that many of the rapid-growing fungi so readily isolated in the manner above noted do not make conspicuous growth in a moist chamber when inoculated upon strips of gray duck without added nutrients, whereas the slow-growing *Leptosphaeria* spp. and *Hendersonia sarmentorum* frequent upon samples of gray duck after long-time exposure to sun and air are more active under these conditions. Since the two species of *Leptosphaeria* growing upon strips of gray duck in pure culture failed to cause as rapid tendering as the isolates of *Hendersonia* and *Diplodia,* it is possible that these two isolates of *Leptosphaeria* are able to derive sufficient nutrition for fruiting without comparable rate of destruction of the cellulose. In this connection it may be significant that the angiocarps of these species were observed occasionally upon those very light-colored areas that showed considerable resistance to puncture. The hyphae of these two cultures were, furthermore, relatively light-colored compared with the hyphae of *Hendersonia* and *Diplodia.*

Since early in the aerial exposure of gray duck there may appear upon it an extensive growth of *Alternaria* sp. and other evanescent Hyphomycetes which soon disappears without notable tendering of the fabric, it is not unlikely that further study may reveal for them a role as agents in predisposing the substratum to ultimate attack by those slower-growing angiocarpous fungi of the type here regarded as the actual cause of decay. Certain it is that the fabric at the time and after the disappearance of this early hyphomycetous sporulation has not been appreciably tendered as revealed by the burst test. Meanwhile until evidence to the contrary is available it must be admitted that these superficial pioneers may be merely a flash growth of little significance in fabric tendering under conditions of aerial exposure only.

Fungal attack on fabric above ground is necessarily quite different from fungal attack during soil contact because of the restricted flora in the former case, characterized by slower activity; in the latter, the agencies may be legion and any mechanical protection natural to the fibers is soon eliminated. The fairly complete mechanical protection provided by the natural coating or an applied coating on the fibers, although not fungicidal, tends to impede fungal attack during aerial exposure, but is of little avail during ground contact.

It has already been pointed out that, with few exceptions, the upper surface of the fabric which has been exposed at a 45° angle facing south shows relatively little evidence by reflected light of fungal attack, in striking contrast with the amount of discoloration to be seen on the lower side, whereas considerably more darkening is revealed by transmitted light. There are probably two main factors governing this unequal distribution of fungal colonies: (1) The lethal radiation of the sun evidently prevents hyphae from growing at or near the upper surface. (2) Moisture requirements of the fungi are met for longer periods on the lower surface, where water collects and evaporation is slowed.

The fact that many of the hyphae are within the lumina of the natural cotton fibers would indicate that much of the nutriment is supplied by the protoplasmic remains, and not necessarily by the chance additional nutrients from dust and plant and animal products.

The results of the sodium hydroxide-carbon disulfide swelling test with fibers from the clearer areas of the gray duck after long-time exposure in Florida would indicate that the primary wall of these fibers had been relatively undamaged by either the biological or non-biological factors of the environment. These results were further substantiated by the burst test data (fig. 18) and the absence of hyphae as shown by microscopic examination. It seems significant that after eighteen months' weathering in Florida these uninfected fibers show relatively little damage as evidenced by the three different types of tests employed: chemical, visual, and mechanical. It is quite likely that solar radiation for the period considered has in itself little effect on the natural fibers owing to their protective coating of waxes and oils.

The data obtained from burst tests (fig. 18) of mineral khaki show that where fungal attack and non-biological breakdown occur side by side on the same piece during aerial exposure, there is no significant difference in resistance to puncture. This is in sharp contrast to the condition in gray duck where after exposure to sun and air any biological breakdown in the uncolored areas is much less readily evaluated than the fungal damage on the same piece

of fabric, which is measurably much more severe. Conceivably, the very darkness of the fungal colony accelerates the breakdown of the fiber structure by increased insolation. However, whether the greater weakening is due solely to the presence of the fungus, or to the fungus plus increased insolation, the fact remains that in this case the fabric at the site of a fungal colony was weakened several fold more than in those portions lacking hyphal penetration. There has hitherto been no adequate method for quantitative differentiation between fungal and non-biological breakdown where these factors are acting simultaneously upon the same fabric. Although similar methods have been utilized in testing fruits, fruit products, etc., this appears to be the first report of such a method applied to fabrics. It should be obvious that this burst testing method has application also for special requirements of fabrics where the traditional fracture techniques are inapplicable.

The physical structure of the cloth may well be of importance in determining the distribution and kinds of fungi. As previously mentioned, species of *Ophiobulus, Leptosphaeria, Hendersonia, Diplodia, Diplodiella,* and *Phoma* are known as leaf and stem fungi, frequently with fruiting bodies erumpent. The tightly woven duck, designed as a water-repellant fabric, is apparently well suited to the growth of these leaf and stem fungi. Many of the angiocarps can be seen actually erumpent among the fibers of an individual yarn in the cloth. Although lighter-weight fabrics have not been studied, it is possible that such materials as sheetings and nettings may present quite a different fungal flora after aerial exposure, even though the material is woven from natural cotton fibers.

Fungal attack of gray duck during aerial exposure in the city of Baltimore was much less in evidence, even after 2¾ years. Viewing by transmitted light and microscopic examination, however, revealed some fungal invasion. Fruiting structures were rare and when found were without identifiable contents. Nevertheless, there was a very considerable tendering of this fabric even though it had been hanging in a vertical position on the north side of the exposure racks. Even more striking was the condition of the sample from the boat cover that had been exposed to weathering for several years in an especially smoky area of Washington, D. C.; although this fabric was very much tendered, no fungal invasion was detected except where the fabric was folded and near the ground. In all probability city smoke with its rich content of sulfur tends to curtail hyphal growth and impede complete development. Lichens, which are composed of fungal and algal constituents, are notoriously absent from buildings, trees, and fences in cities.

It might be contended that angiocarpous fungi of the type here considered represent a late stage in a microbial succession, a point of view that would well be worth critical investigation. For the present it suffices to reiterate that after aerial exposure of six months or more, angiocarps are occasionally present on those very areas with least discoloration, i.e., those whitish spots which show the greatest resistance to rupture. This may be an expression of a localized retardation of growth and development or possibly merely the result of accidental late infection of the area. Certainly in these white areas with but few angiocarps there are very few fibers containing hyphae.

The fact that the isolates made from angiocarps obtained from these fibers were able to weaken strips of gray duck without additional nutrients in a saturated atmosphere would suggest that additional isolates of some other angiocarpous fungi might perform similarly. Furthermore, the weakened dark fungal spots on the fabric with their erumpent and superficial angiocarps are convincing evidence in themselves that the mycelia associated with them are the main cause of decay.

It is very probable that the low viability and abortion of so many of the spores of these angiocarps are the result of the lethal action of solar radiation combined with long periods of desiccation alternating with short wet periods.

The British workers (Galloway and Burgess, 1940) have coined the phrase "mildew capacity" with reference to the varying amounts of food materials present and capable of supporting fungal growth on fabrics. Obviously, gray duck, which consists of the natural fibers with adherent fragments of seed-coats, etc., spun and woven, sometimes plus a sizing, would have a much higher mildew capacity than the fibers alone. Similarly, bleached duck, depending, of course, on the degree of bleaching and scouring, should have the least capacity for the support of fungal growth. Part of this capacity to support fungal growth is now known to be due to the presence of vitamins B_1, B_6, and biotin (Robbins and Ma, 1942), as well as other substances possibly capable of satisfying nutritional requirements in minute amounts (Robbins and Schmitt, 1945), some of which are doubtless lost in the bleaching process.

The present study can be regarded as a pioneering attempt to determine the etiological relations of one type of fabric tendering and it is to be expected that more intensive investigations will yield added information to this end. Use of new technical approaches to the problem should be productive of a more adequate understanding of the nature of deterioration of cellulosic materials, leading to practical methods of prevention or control.

SUMMARY[3]

Certain angiocarpous fungi heretofore unappreciated and unrecognized as being primarily involved in the decay of gray duck or in the decomposition of cellulose are here reported as significant agents in

[3] The points of view here expressed, relative to the deterioration of sun-exposed duck by angiocarpous fungi with dark hyphae, are further supported by certain field and laboratory observations made in Panama by Zuck in 1945 as well as by some preliminary studies by Diehl in 1944 of specimens after weathering in the South Pacific war zone.

the slow cellulosic breakdown of fabric during aerial exposure. They are *Diplodiella Cowdellii, Hendersonia sarmentorum, Hendersonia* sp., *Leptosphaeria* spp., *Diplodia* sp., *Phoma herbarum,* and *Ophiobolus* sp.; other angiocarpous fungi present but unidentified may also be of significance. These are regarded as representatives of the normal saprophytic flora of dead stems and leaves of plants.

It is considered that these angiocarpous fungi have tended to escape detection for two reasons: (1) The employment of standard plating techniques which do not detect these slow-growing fungi. (2) Samples of gray duck develop these angiocarps only after long periods of aerial exposure.

When fabrics are exposed at a 45° angle facing south the angiocarps of these fungi develop ordinarily only on the shaded surface. Consideration is given to the factors regarded as significant to this phenomenon.

The limited data here obtained reveal a considerable tendering in the absence of fungal hyphae in the case of mineral khaki exposed in Florida and in the case of gray duck after long exposure to the smoky atmosphere of two northern cities.

A quantitative method is given for evaluating fungal damage as distinct from non-biological damage when these occur simultaneously on the same fabric. It is suggested that this method is also applicable generally to testing localized differential resistances to puncture and bursting which is not possible by the techniques generally employed.

Numerous fibers from the clear areas with exposures of from three to eighteen months when treated by the sodium hydroxide-carbon disulfide swelling test disclose the beaded phenomenon.

The combined evidences of visual, chemical, and mechanical tests demonstrate that certain areas of fabrics even after eighteen months aerial exposure may remain relatively undamaged.

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PRELIMINARY EXPERIMENT ON THE CULTIVATION OF BAERIA CHRYSOSTOMA UNDER STERILE CONDITIONS [1]

Shih-Wei Loo[2]

BY GROWING plants under sterile conditions certain problems such as the effect on the growth of plants of various organic substances and nitrogen compounds which are readily attacked by bacteria may be studied in a simplified and controlled way. Attempts have been made by different workers to grow plants under sterile conditions, but there is no record of any higher plant which has been successfully cultured for a complete life cycle under sterile conditions. Klein and Kisser (1924, 1925) developed many elaborate devices for growing young plants in sterile culture. Harrison and Barlow (1907), Grijns (1927), Wilson and co-workers (1931a, 1931b) and Virtanen and collaborators (1935, 1936, 1939), who were interested in the problem of nitrogen fixation, have grown leguminous plants with *Rhizobia.* Molliard (1907, 1915), Rischkow and Bulanowa (1931), Knudson (1916, 1920, 1933), Knudson and Lindstrom (1919), and Brannon (1923) studied the effect of various organic substances, especially carbohydrates and amino acids, on the growth of various plants. It is also interesting to note that Knudson (1930) attempted to grow a hybrid orchid (*Laelia-Cattleya*) to the flowering stage under aseptic conditions. The orchid did produce flowers after a period of growth (1923–1928) and three transfers into fresh culture medium, but the culture was found to be badly contaminated. The author re-

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[2] The writer wishes to express his gratitude to Prof. F. W. Went for his valuable suggestions and criticisms throughout the whole course of the work. This work was carried on at the William G. Kerckhoff Laboratories, California Institute of Technology, Pasadena 4, California.

ported that the following organisms were found in the culture flask: one species of *Chlorella,* two forms of *Penicillium,* one *Fusarium,* two forms of yeast, several bacterial colonies and one colony of moss. He also pointed out that none of the fungal hyphae observed had the characteristic appearance of the orchid symbiont, and concluded that obligative symbiosis is not requisite either for germination or for flower production of this orchid. Brannon (1923) reported that pea plants growing in the dark "produced rudimentary flowers, which, however, became abscissed in the course of a few days." Rudimentary flowers produced in etiolated condition are not a normal phenomenon and they certainly do not constitute a complete life cycle. Gentcheff and Gustafsson (1940) claimed that pea plants were grown from seed to seed in agar medium but they did not present evidence showing that the plants were actually growing in completely sterile conditions. In the cases mentioned above, the investigators were not able to maintain the plant in sterile culture long enough for completion of the life cycle. One difficulty in completing the growth cycle under sterile conditions may have been the limited capacity of the culture vessels which must necessarily be used. For example, Knudson and Lindstrom (1919) and Knudson (1930) had to use culture tubes as large as 500 × 600 mm. in order to grow albino corn or other plants, and 12-liter flasks to grow orchids. Other authors grew plants in such a way that only the root system was maintained under sterile conditions leaving the aerial portion exposed to air (Knudson, 1916, 1920; Knudson and Lindstrom, 1919; and Spoehr, 1942, etc.).

It was found in this laboratory by Sivori and Went (1944) and Lewis and Went (1945) that several California desert annuals flower at a small size after only a short period of vegetative development. Among eighty annuals tested, it was observed that *Baeria chrysostoma* grew best and flowered first under controlled non-sterile conditions. Because of the small size of the plant at the time of flowering and seed setting, attempts were made to grow *Baeria* in sterile culture. This small plant has the advantage that it can be grown in small test tubes, and a large number of plants can be conveniently studied in one experiment. This report presents a part of the experimental data obtained by growing *Baeria* in sterile culture.

MATERIALS AND METHODS.—*Baeria chrysostoma* T. & G. was used exclusively throughout this work. It is an annual plant which flowers during March and April, native to the Pacific Coast at elevations below 2500 feet throughout California and southern Oregon. It is usually branched at the base and 100 to 250 mm. in height. Sivori and Went (1944) showed that as few as 19 days may elapse between germination of the seed and flowering.

Seeds of *Baeria*[3] usually germinate poorly unless they are first soaked and washed. When seeds were directly sown in pots, germination was about 10 per cent compared to 90 to 95 per cent germination of seeds which had been soaked and washed for two hours previous to placing them in germination dishes. The seeds were disinfected before germination by washing with several changes of distilled water over a period of two hours, followed by soaking in 0.1 per cent bichloride of mercury for 8 to 10 minutes, again followed by at least five 10-minute washings with sterile, distilled water.

For germination, soaked and disinfected seeds

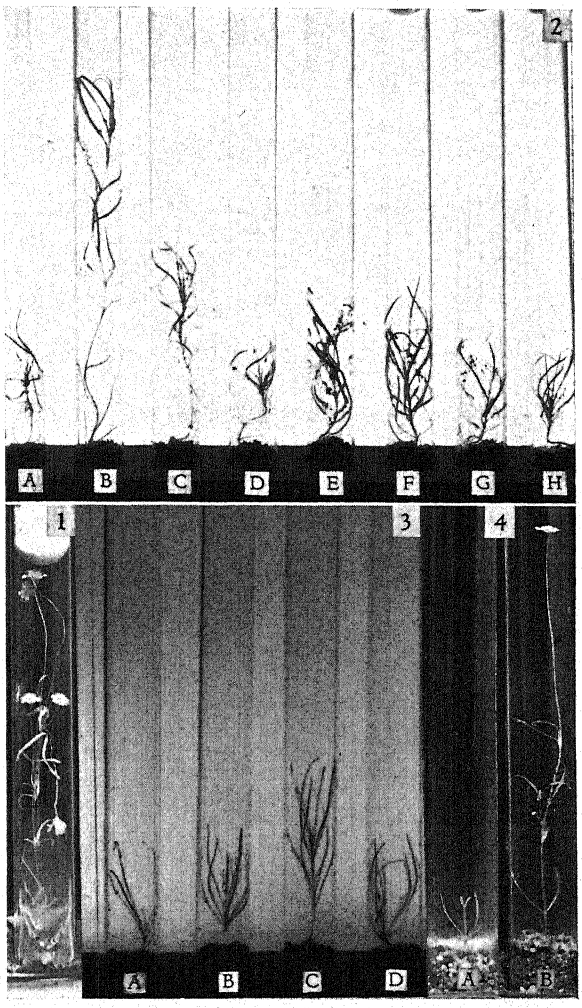


Fig. 1–4.—Fig. 1. *Baeria* grown on agar medium for 90 days in continuous light at 17°C. (Exp. C-18).—Fig. 2. The effect of day and night temperature on the growth of *Baeria;* day temperature, A. to D 26°C.; E to H 17°C.; night temperature, A & E 8°C., B & F 13°C., C & G 16°C., D & H 22°C. (cultures 102 days old, Exp. C-22).—Fig. 3. The effect of sugar on the growth of *Baeria;* A, 0%; B, 1%; C, 2%; and D, 4% sucrose (cultures 50 days old, Exp. C-24).—Fig. 4. *Baeria* grown at 17°C. for 43 days in normal daylight (A) and in continuous light (B), (Exp. C-21).

[3] The seeds used for this study were obtained from the Rancho Santa Ana Botanic Garden, Fullerton, California.

TABLE 1. *Effect of different day and night temperatures on the growth of Baeria in culture tubes containing 20 cc. of washed sand, 8 cc. of nutrient solution. Ten plants in each treatment (Exp. C-22-1).*

Day temperature, °C.	17				26			
Night temperature, °C.	8	13	16	22	8	13	16	22
Pairs of leaves (average)	12.0	13.5	12.0	11.0	11.6	14.0	13.2	14.9
Width of leaves in mm. (average)	1.8	1.3	1.0	0.8	1.5	1.4	1.1	0.8
Height of top in mm. (average)	94.5	103.5	59.0	55.0	129.4	207.2	99.0	79.3
Fresh weight of top in gm. (average)	0.36	0.47	0.20	0.16	0.18	0.23	0.13	0.10
Length of internodes in mm. (average)	....	....	....	....	9.1	16.9	8.8	3.6

were sown on 0.5 per cent agar containing half strength Hoagland's solution. An agar medium was employed since the small seeds can be separated more easily on agar than on filter paper and any bacterial contamination is easily detected. The germination dishes were placed in the air-conditioned greenhouse of this Institute (Went, 1943, 1944) at 17°C., germination occurring in about one week. Uniform and vigorous seedlings, 5 to 10 mm. in length, were transplanted aseptically to individual culture tubes. Fifteen plants were started for each treatment; these were kept in normal daylight at 17°C. in the greenhouse. After two to three weeks when the plants had become established, ten plants were selected for each treatment on the basis of uniformity and approximately equal length (20 to 30 mm. long and with three pairs of leaves). Each group was then subjected to a specific treatment as to photoperiod, temperature, etc. The length of shoot, the number of leaves and number of flowers were used as criteria of growth.

All experiments were carried out in the air-conditioned greenhouse. Artificial light was supplied by fluorescent bulbs of about 450 foot-candles intensity.

In all of the experiments, 300 × 25 mm. culture tubes were employed. Each tube contained 20 cc. of washed, sterilized sand of 8 to 20 mesh and 8 cc. of Hoagland's solution (Hoagland and Arnon, 1938) with or without the addition of supplementary substances. The tubes were plugged and autoclaved for 20 minutes at 15 pounds.

It was found that *Baeria* plants in sterile culture grew equally well with their roots growing in sand or in 1 per cent agar medium. Figure 1 is a photograph showing a plant grown on agar medium for 90 days in continuous light at 17°C. Some of the flowers had already withered in the tube.

GROWTH AFFECTED BY DAY AND NIGHT TEMPERATURES.—Lewis and Went (1945) showed that day and night temperatures have a profound influence on the growth as well as on the development of *Baeria*. They reported that the day temperatures employed did not produce any difference on flower initiation, size of leaves or rate of leaf production. However, night temperatures had a pronounced effect on the rate of leaf production and also on leaf size. The higher the night temperature, the greater the rate of leaf production. A series of preliminary experiments was made in which various combinations of day and night temperatures were used to see if this phenomenon was true when *Baeria* was grown in sterile culture. The plants received an 8-hour photoperiod of normal day light each day and were maintained for a total of 122 days. During this period they were subjected either to a 17°C. or to a 26°C. temperature for the 8-hour photoperiod. Separate groups were transferred daily at the end of the photoperiod to dark rooms maintained at 8, 13, 16 or 22°C. for the 16-hour dark period. Table 1 and figure 2 show that maximum growth occurs when *Baeria* is maintained at a day temperature of 26°C. and a night temperature of 13°C. At the lower day temperature, 17°C. also, the plants grow best at a night temperature of 13°C. though the total growth is only about 50 per cent of that of the plants grown under the higher day temperature. The total number of leaves formed by *Baeria* is apparently unaffected by differences in day and night temperature, but the width of the leaves increases with decreasing night temperature. The fresh weight of the shoots is greatest at a 13°C. night temperature, the same temperature where optimal growth in length occurs. At a day temperature of 26°C. growth in height is paralleled by the length of the internodes.

TABLE 2. *Effect of sugar on the growth of Baeria in culture tubes containing 20 cc. of washed sand, 8 cc. of nutrient solution. Ten plants in each treatment (Exp. C-24-1).*

Average height in mm. of top at	Per cent of sucrose			
	0	1	2	4
25 days	15.0	22.5	37.0	21.4
42 days	30.7	37.5	61.0	38.0
61 days	45.7	52.0	69.8	50.7

GROWTH AS AFFECTED BY SUGAR AND NITROGEN.—Preliminary experiments showed that when sugar was added to the culture medium the growth of *Baeria* was improved. Attempts were made to determine the optimal sucrose concentration. The plants were grown for 61 days at a constant temperature of 17°C. and were given 8 hours of daylight. The results of one such experiment are shown in table 2 and also in figure 3. Sugar increased the growth of *Baeria*, the optimal concentration being about 2 per cent. Results of a later experiment discussed

TABLE 3. *Osmotic value and sugar content of Baeria grown in an 8-hour day in various concentrations of sugar (Exp. C-24).*

Culture number	Sucrose concentration in nutrient		Osmotic value of leaf cells (in molar concentration of sucrose)	Percentage of sucrose (dry weight basis of whole top)
	percentage	molar		
2	0	0	0.29 M.	0.77
5	1	0.029	0.32 M.	1.34
9	2	0.058	0.34 M.	1.10
14	4	0.116	0.39 M.	1.44

below (table 5) show that sugar has a striking effect in promoting the growth of this plant.

Osmotic values of the leaves and the sugar content of the shoots were determined. The osmotic values of the leaf cells were determined by the plasmolytic method. The results are summarized in table 3. It is interesting to note that the osmotic value of the leaves increased with an increasing concentration of sugar in the nutrient. However, the sucrose content of the plant tissues, determined by the ceric sulfate method (Hassid, 1937), did not show such a linear relationship although the concentration found in the control leaves was lower than that found in the plants grown in the presence of sucrose.

The availability of various nitrogen-containing compounds for growth of *Baeria* under sterile conditions was studied. An equivalent amount of nitrogen of the various nitrogen compounds was used to replace the nitrate nitrogen in the Hoagland's solution. The composition of the nutrient and experimental results are given in table 4. In all respects, nitrate nitrogen was the best source for the growth of *Baeria*. Plants supplied with nitrate were taller and produced more leaves and flowers than those plants grown in nutrient supplied with nitrite, ammonia or organic nitrogen. The results also showed that *Baeria* was able to utilize urea to a considerable extent. All of the other nitrogen-containing substances depressed the growth of *Baeria*.

Photoperiod and sugar.—Sivori and Went (1944) have reported that *Baeria* is photoperiodically sensitive, requiring a minimum photoperiod of 15 hours for flowering. Lewis and Went (1945) later found that the photoperiod of *Baeria* changes with age. Young plants are typically long day plants. As the plant ages, it becomes indifferent to the photoperiod. They found that *Baeria* remained a long day plant for not longer than 40 days. The light intensity required as a supplement to the 8-hour natural daylight to make *Baeria* flower is fairly high (more than 100 f.c.). Since red light is most, blue less and green not effective, it was conceivable that this photoperiodic effect on young plants was the result of deficient synthesis of carbohydrates during the 8-hours of light available for photosynthesis, and that the amount of carbohydrate in turn limits the flowering response. If this were the case, the photoperiodic response of young *Baeria* plants could be overcome by adding an artificial supply of sugar.

In one experiment, *Baeria* was grown in culture tubes with or without the addition of 2 per cent sugar to the culture medium. The plants were germinated on March 27, 1945, transplanted into test tubes and placed in the greenhouse in normal daylight at 17°C. on April 2. From April 26, 1945 on, the plants were kept at a constant temperature of 17°C. and subjected to photoperiods of 4, 6, 8, 10, 12, 14, 16 or 18 hours of artificial illumination per day. The results of this experiment are summarized in table 5. The data show clearly that sugar added to the medium greatly promoted the growth but did not markedly effect the photoperiodic response

TABLE 4. *Effect of various nitrogen sources on the growth of Baeria in culture tubes containing 20 cc. of washed sand, 8 cc. of nutrient solution, at 17°C. The first 17 days in daylight, subsequent 47 days in artificial, continuous light. Ten plants in each treatment (Exp. C-25).*

Culture number	cc. of M/10 solution per liter added to replace NO_3 in Hoagland solution		Pair of leaves (average)	Average length of shoot (mm.)	Average number of flowers	No. of plants surviving after 64 days culture
1	$Ca(NO_3)_2$, control	50	6.6	230	4.1	10
2	$NaNO_2$	100	0.0	0	0.0	0
3	NH_4Cl	100	4.8	84	1.0	5
4	Urea	50	5.7	100	2.2	10
5	Asparagine	50	4.0	10	0.0	1
6	Glutamine	50	3.5	13	0.0	2
7	Peptone	0.091%	4.6	32	0.6	5[a]

[a] One plant exceptionally long and only this plant produced flowers.

TABLE 5. *Effect of photoperiod and sugar on the growth and development of Baeria in culture tubes containing 20 cc. of washed sand, 8 cc. of nutrient, with or without the addition of 2 per cent sucrose at 17°C., under artificial light. Ten plants in each treatment (Exp. C-26).*

	Photoperiod	4 hours		6 hours		8 hours		10 hours		12 hours		14 hours		16 hours		18 hours	
	Date	% sugar 0	2	0	2	0	2	0	2	0	2	0	2	0	2	0	2
Average number of pair of leaves	5–13–45	3.2	4.3	3.4	4.6	4.0	5.1	3.4	5.1	4.2	5.5	4.7	5.7	3.2	6.1	3.0	6.4
	20	3.5	4.6	4.1	5.1	4.3	5.6	4.1	5.6	5.3	6.5	5.7	7.1	3.8	6.9	3.1	7.4
	26	3.6	4.7	4.6	5.2	4.4	5.7	4.4	5.8	5.4	6.8	5.8	7.4	4.4	7.5	4.0	7.4
	6–3	4.0	5.4	4.6	5.8	5.3	6.8	6.5	7.4	6.5	8.7	7.0	8.5	4.6	8.3	5.5	7.4
	17	5.0	6.7	6.2	8.1	7.4	9.9	8.5	10.2	8.1	10.8	9.0	10.6	5.0	8.3	5.5	7.4
	7–8	...	...	...	...	...	...	8.5	10.2	9.3	13.0	9.9	10.6	5.0	8.3	5.5	7.4
	22	8.0	9.3	9.3	11.1	10.0	13.2										
	8–5	9.0	11.3	10.6	13.0	11.1	15.6										
	19	9.0	11.6	11.0	14.4	12.3	16.1										
	9–16	9.0	13.5	11.0	16.9	14.0	17.6										
Average length of top (in mm.)	5–13–45	21.6	33.5	24.3	33.0	28.6	45.5	27.9	47.2	38.5	55.5	42.8	51.5	23.0	69.4	16.4	82.2
	20	23.3	33.5	24.3	35.0	35.0	51.0	32.8	51.7	47.1	59.0	49.3	55.0	29.0	93.9	23.3	110.5
	26	26.2	37.8	26.7	38.2	38.6	51.2	42.4	59.8	54.3	70.3	59.7	65.9	46.6	132.7	43.3	170.0
	6–3	27.2	37.8	26.7	39.2	38.6	56.1	55.0	59.8	62.8	73.4	61.4	71.5	55.0	146.4	51.6	186.6
	10	29.0	38.3	26.7	40.5	42.1	58.2	55.0	65.0	...	...	74.3	78.0	74.0	173.3	71.6	195.5
	17	29.0	42.0	33.7	46.6	44.2	58.9	57.5	70.5	77.1	85.0	88.0	93.9	85.0	184.4	95.8	202.2
	24	30.5	42.0	34.5	51.1	51.3	65.5	88.0	76.4	83.3	94.6	101.4	113.7	100.4	198.8	116.3	206.0
	30	33.2	42.0	38.0	52.5	53.4	69.5	89.5	80.2	84.1	109.3	114.0	117.3	103.6	201.3	121.5	208.0
	7–8	35.0	42.0	38.0	56.0	55.7	71.0	89.5	83.5	85.0	112.8	110.7	120.6	108.0	201.3	121.5	208.0
	22	40.0	45.5	39.0	58.0	65.5	...										
	8–5	40.0	46.3	39.0	62.4	70.7	106.2										
	19	40.0	47.1	40.0	70.8	79.3	125.6										
	9–16	40.0	58.7	40.0	81.5	79.3	142.8										
Number of flowers per number of plants survived	5–13–45	0/6	0/10	0/7	0/10	0/7	0/10	0/7	0/9	0/7	0/9	0/7	0/10	0/6	1/9	0/7	3/9
	20	0/6	0/10	0/7	0/10	0/7	0/10	0/7	0/9	0/7	0/9	0/7	0/10	1/5	4/9	1/6	11/9
	26	0/5	0/9	0/7	0/10	0/7	0/10	0/7	0/9	0/7	0/9	0/7	0/10	1/5	6/9	1/6	17/9
	6–3	0/5	0/9	0/7	0/10	0/7	0/10	0/2[a]	0/9	1/7	0/9	0/7	2/10	4/5	20/9	6/6	24/9
	10	0/5	0/9	0/7	0/10	0/7	0/10	0/2	1/9	1/7	0/9	1/7	7/10	4/5	21/9	7/6	30/9
	17	0/4	0/7	0/4	0/10	0/7	0/9	0/2	1/9	1/7	0/9	3/7	12/10	5/5	30/9	8/6	39/9
	24	0/4	0/7	0/4	0/10	0/7	0/9	0/2	2/9	1/7	0/9	4/7	15/10	7/5	31/9	13/6	39/9
	30	0/4	0/7	0/4	0/10	0/7	0/9	0/2	2/9	1/7	1/9	5/7	15/10	9/5	34/9	13/6	43/9
	7–8	0/2	0/7	0/4	0/10	0/7	0/9	0/2	2/9	2/7	5/9	12/7	22/10	11/5	42/9	20/6	53/9
	7–22–45	0/1	0/6	0/3	0/10	0/7	3/9										
	8–5	0/1	0/6	0/3	0/10	0/7	7/8										
	19	0/1	3/6	0/1	5/9	2/7	15/8										
	9–16	0/0	13/4	0/1	13/8	2/4	19/7										

[a] Five plants in this series were lost due to an accident.

of the plants. However, plants subjected to 4 and 6 hours light that received no sugar failed to flower even when maintained in culture for a long period of time, but those which received sucrose did ultimately flower. In the case of 8, 10, 12 and 14 hours illumination, relatively fewer flowers were produced by the plants grown in medium without added sugar as compared to those grown in medium with additional sugar. At the 4-hour photoperiod most of the plants receiving sugar ultimately started to flower, but in two plants the flower initiation was not followed by further differentiation. The stem was terminated by a whorl of about eight short leaf-like organs, which obviously were initiated as involucral bracts. Macroscopically no further differentiation of the growing point was visible. Figure 4 is a photograph from a parallel experiment showing a typical plant flowering at day length of 24 hours contrasted to a plant grown under conditions of normal day length.

As has been shown, plants placed under continuous illumination begin to flower after about 30 days and later set seed. In this way, it was possible to grow *Baeria* under absolutely sterile conditions from the beginning to the end of its normal life cycle. Twenty-four seeds were harvested from a sterile culture, which gave rise to two seedlings. The low percentage of germination may be due to failure of pollination or failure of the fertilized seeds to mature properly.

Discussion.—It has been shown that *Baeria chysostoma* is able to grow to maturity and produce seeds under sterile conditions but that the optimum growth rate depends on a correct combination of day and night temperatures. Low night temperature of 13°C. in combination with day temperatures of either 26° or 17° gave the best growth as shown in table 1, although the total growth at 17°C. was only about half of that at 26°C. Lewis and Went (1945) found that the growth and the production and size of leaves of *Baeria* was accelerated by high night temperatures but not influenced by different day temperatures. In the present case, however, the best growth (length and fresh weight) was obtained with a comparatively low night temperature and a high day temperature. Furthermore, variation in day and night temperatures was found to be without effect on the production of leaves although the width of leaves was increased with an increase in night temperature, in accordance with the findings of Lewis and Went.

It has been found by a number of authors that sugar favors the growth of higher plants. Molliard (1907, 1915) showed that sugar promoted the growth of radish and other plants. Knudson (1916) and Knudson and Lindstrom (1919) reported that sugar favored the growth of corn and radish. Brannon (1923) found that pea, alfalfa, radish and timothy can greatly increase their dry weight when grown in the dark on nutrient solution containing glucose and fructose. As early as 1889 Saposchnikoff reported that starch formation occurred in the dark in plants supplied with sugar.

In the present work with *Baeria,* two per cent was found to be the optimal sugar concentration. In general, sugar accelerated the growth of the plants. It is also interesting to note that when sugar was supplied in the nutrient the plant developed a higher osmotic value than the controls. This phenomenon was observed also by Wildervanck (1931, 1932) in *Nitella* plants. In the case of *Baeria,* the control plants had an osmotic value of 0.29 M (in terms of sucrose) while in the plants treated with 1 to 4 per cent sugar, the osmotic value increased to a figure as high as 0.39 M. Such values are much higher than the sugar concentration used in the nutrient medium. The increased salt content in plants grown in nutrient solutions of high osmotic value is a similar phenomenon which has been reviewed by Magistad (1945).

Nitrate nitrogen is generally recognized as the best nitrogen source for green plants.[4] Ammonium nitrogen can replace nitrate nitrogen, but in most cases it is inferior to nitrate, as has been shown by Krüger (1905), Maze (1898, 1900), Prianischnikow (1908, 1923), Stewart (1925), Hutchinson and Miller (1909), etc. The same is true for *Baeria* grown in sterile culture. When ammonium chloride was supplied as the only nitrogen, many of the plants died and only one plant flowered. Nitrite is usually considered as an unavailable source of nitrogen, although Schultz (1903) and Mevius and Dikussar (1930) found that very dilute solutions (about 50 mgs. to 100 mgs. per liter) were capable of supporting the growth of some plants. An 0.01 M solution of nitrite was apparently toxic to *Baeria* plants, since no plants survived after transplanting to the culture tubes.

Pirschle (1929), Yamaguchi (1930) and Tanaka (1931) showed that urea can be utilized directly by a number of plants. This is also true for *Baeria,* which was able to grow and flower in the presence of urea as the only source of nitrogen, although growth measurements showed it to be inferior to nitrate. In regard to the availability of other organic nitrogen compounds, Klein (1930) reported that glutamine and aspartic acid can be absorbed directly. Knudson (1933) found, however, that glycine, leucine and aspartic acid were not utilized. *Baeria* showed very poor growth and a very poor survival when fed with asparagine, glutamine and peptone. On the whole, nitrate was the best source for the growth and development of *Baeria.*

SUMMARY

It has been shown that *Baeria* plants are able to grow to maturity and produce seeds under aseptic conditions.

Low night temperatures produced the best growth in *Baeria* irrespective of the temperature used during the day. The best growth was obtained with a

[4] For extensive literatures, reference may be made to Nightingale (1937).

day temperature of 26°C. combined with a night temperature of 13°C. The production of leaves was not affected by different day or night temperatures. The width of the leaves increased with an increase in night temperatures.

Two per cent sugar added to the nutrient improved the growth of *Baeria*. "Osmotic adaptation" of the cells apparently occurred.

Nitrate nitrogen was found to be the best nitrogen source, but urea was utilized to a considerable extent by *Baeria*.

Long photoperiod and added sugar accelerated the initiation and production of flowers of *Baeria*. These factors had a significant effect on the development of young plants. However, *Baeria* produced flowers also under short day conditions, provided they were kept in culture long enough.

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A MORPHOLOGICAL, DEVELOPMENTAL, AND CYTOLOGICAL STUDY OF FOUR SAPROPHYTIC CHYTRIDS. III. CATENOCHYTRIDIUM LATERALE HANSON [1]

Anne Marie Hanson

CATENOCHYTRIDIUM LATERALE Hanson occurs as a saprophyte on decaying vegetable debris in soil collected near Sharon, Connecticut. It may be cultured on cellophane in soil extract, in exactly the same manner described for *Catenomyces persicinus* (Hanson, 1945). Unlike the latter, its growth is as vigorous on onion skin, corn and grass leaves as on cellophane. On cellophane, however, the growth rate is slower than on the other substrata, the cycle requiring ten to fourteen days, while on the other substrata it requires only five to eight days. As was reported previously by the author (1944), this fungus is very similar to *Catenochytridium carolinianum,* the type species of the genus, described by Berdan (1941). It differs from the latter species, however, by its smaller multiguttulate zoospores, by the asymmetrical position of the sporangium, and the presence of a predominant primary apophysis. The type of development of the thallus, on the other hand, is endo-exogenous like that of *C. carolinianum* and *Chytridium Lagenaria* (Karling, 1936).

Structure and germination of the zoospore.—The living zoospore is hyaline, spherical (2.9–4.5 μ), and usually multiguttulate. Typically one refractive globule is much larger than the others and occupies a central position in the spore (fig. 1, 2). The nucleus cannot be seen in the living spore, but when spores are fixed and stained, it appears as a relatively clear spherical area posteriorly in the spore body (fig. 3). It is surrounded anteriorly and laterally by a densely staining nuclear cap.

The active swimming period of the spores lasts from twenty minutes to approximately one hour, at the end of which they usually become amoeboid. If the spores come to rest on a favorable substratum, most of them germinate. In so doing, they produce a relatively stout germ tube which penetrates the substratum to establish the intramatrical absorptive system.

Differentiation of the germ tube into a "primary apophysis" and rhizoids takes place usually after the germ tube has branched, but in rare instances the apophysis appears to develop prior to the formation of branches (fig. 4). In the first instance, the apophysis may develop in one of four ways: (1) as a swelling immediately above the point of origin of branches (fig. 5, 6), as in *Chytridium Lagenaria* (Karling, 1936); (2) as a basal outgrowth of the germ tube between two opposite branches (fig. 7), as in *Diplophlyctis intestina* (Karling, 1930); (3) as a swelling of the germ tube below the region of branches (fig. 8), or (4) as an enlargement in the region of branches (fig. 9). The first three types of development occur in all substrata, but the fourth type is seen only in cellophane which is more resistant to infection than grass leaves and onion scales. The initial slow growth of rhizoids may, therefore, be due to difficulty in penetration of the substratum.

The same relative position of primary apophysis and rhizoids noted in the germination stages is maintained during further growth and development. Figure 10 shows an advanced stage of a rhizoidal system of the type noted previously in figures 5 and 6, while figure 11 shows an advanced stage of a rhizoidal system initially like that shown in figure 7. Obviously, then, the type of origin of the primary apophysis determines the configuration of the fully developed absorptive system.

Whether or not the swellings in the rhizoids are to be regarded as constituting a compound apophysis is a matter of interpretation. If they are regarded as such, then some thalli of *C. laterale* which develop extensive catenulate swellings on the rhizoids during growth of the latter are similar to those of *C. carolinianum.* Such swellings, however, are frequently very reduced or lacking in *C. laterale* (fig. 11, 15), so that the intramatrical system is more like that of a species of *Chytridium.* It may thus be said that the rhizoidal system of *C. laterale* is compoundly apophysate or monoapophysate. The two types occur in equal ratios, and accordingly our fungus appears to be intermediate between *Catenochytridium* and *Chytridium* as regards the organization of the rhizoidal system. The predominant primary apophysis of *C. laterale* also compares more favorably with the subsporangial swelling of *Chytridium* species than *Catenochytridium carolinianum,* for in the latter this structure may be subordinate to secondary swellings. The orientation of the rhizoids on the subsporangial swelling of *C. laterale* as shown in figures 12, 14, and 16 differs, however, from both *C. carolinianum* and *Chytridium*

[1] Received for publication February 6, 1946.

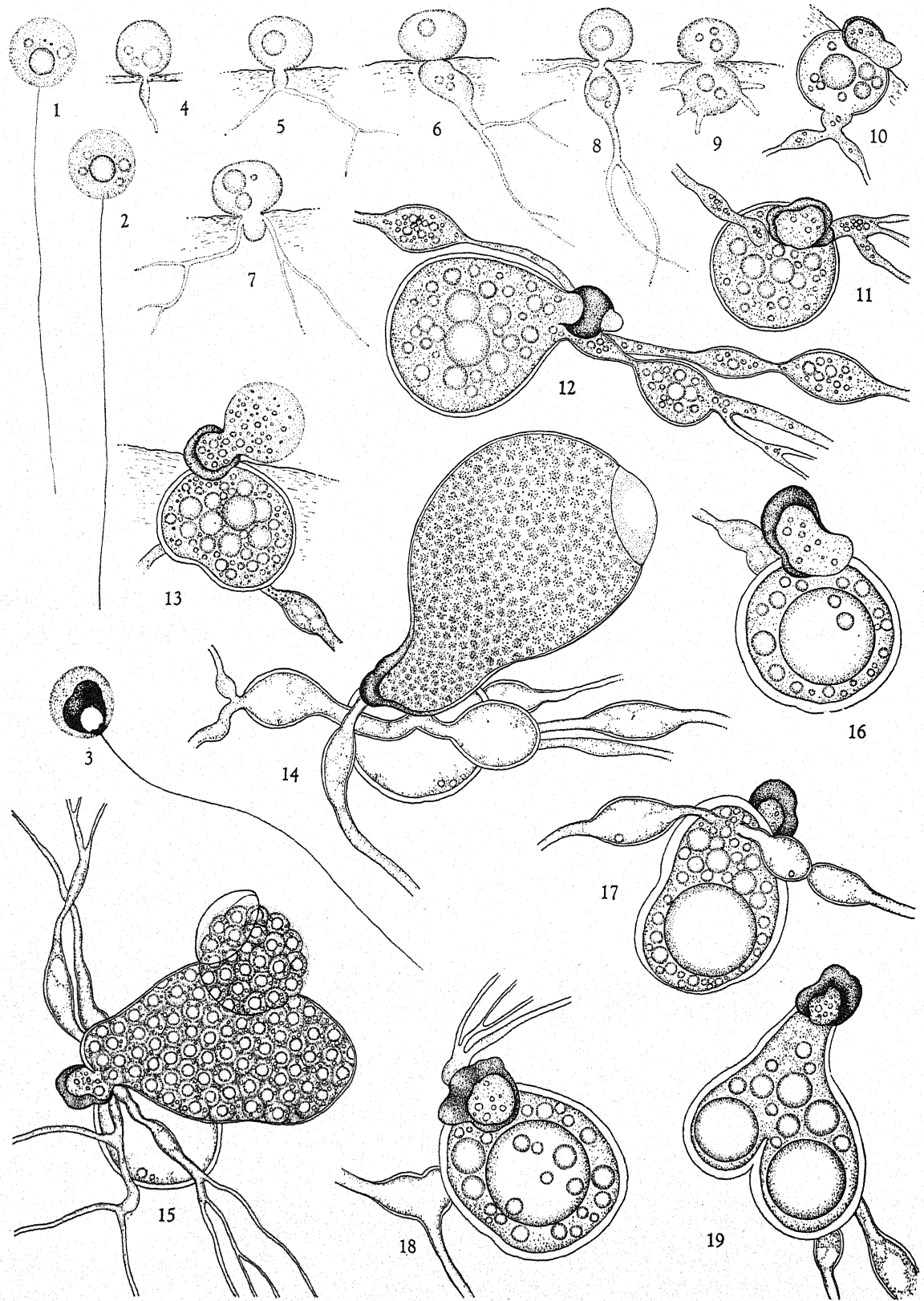


Fig. 1–19.—Fig. 1, 2. Living zoospores. ×5040.—Fig. 3. Stained zoospore showing nuclear cap. ×5040.—Fig. 4–9. Germination stages. ×5040.—Fig. 10. Later stage in the development of an intramatrical absorbing system initially

species, for in these forms the rhizoids have never been shown to be oriented on the apophysis in the region immediately below the attached zoospore cyst.

Development of the zoosporangium.—Subsequent to the development of the intramatrical absorptive system and maturation of its protoplasmic contents in the orthodox *Chytridium* manner, exogenous development begins with the budding out of a small hyaline blister or vesicle from the zoospore case. Usually the bud arises laterally near the base of the zoospore as in *C. aggregatum* (Karling, 1938), *C. Schenkii* and *C. gibbosum* (Scherffel, 1902, 1926). With continued upward movement of accumulated protoplasm in the apophysis and rhizoidal system, the incipient sporangium increases in size, but the zoospore case remains in its lateral position, as is shown in figures 14 and 15. Consequently, the mature sporangia are similar in appearance to those of *Chytridium aggregatum,* which bear basolaterally a yellow or brown zoospore case, and less like those of the type species, *Catenochytridium carolinianum,* in which the zoospore case is usually carried up on the developing sporangium.

At maturity the sporangium dehisces by a large, saucer-shaped operculum, and the zoospores ooze out (fig. 15). As in *C. carolinianum,* the operculum is generally persistent for some time after spore discharge. The emerged spores lie quiescent in a globular mass for a short time. Gradually they begin to move and separate, and before long they become very active and swim away.

Development of resting spores.—At the time this fungus was diagnosed in 1944, resting spores had not been seen, but since that time such structures have been found twice. They developed only sparingly and were not observed to germinate. The initial developmental stages of thalli which give rise to resting spores cannot be distinguished from those developing zoosporangia. However, when the primary apophysis has reached its greatest dimensions, an accumulation of refractive material within it and a thickening of its walls indicates its destiny as a resting spore. As development proceeds, some refractive globules coalesce to form one or two enormous refractive bodies, but the remainder persist as smaller refractive globules around the larger ones (fig. 16 to 19). While fusion of globules is taking place, the wall of the primary apophysis further thickens and becomes golden in color. The protoplasm in which the refractive bodies are imbedded, however, remains hyaline.

If, as it appears, these resting bodies are formed without sexual fusion of gametes, they are nothing more than the encysted primary apophysis. Secondary swellings on the rhizoids of *C. laterale* have never been noted to develop into resting spores as in *C. carolinianum.*

The relation of nuclei to development.—In view of the fact that an extensive system of rhizoids and one or more apophyses develop within the substratum before there is any marked evidence of extramatrical growth, the relation of the nuclei to this endo-exogenous type of development is significant. In his study of living material of *Chytridium Lagenaria,* Karling (1936) postulated that in this type of development the zoospore nucleus might migrate down through the germ tube into the apophysis and there multiply as endogenous growth progressed. Then after this had been completed, the nuclei would migrate out with the remainder of the protoplasm into the persistent zoospore case and form the extramatrical sporangium. In light of this probability, preliminary cytological studies of fixed and stained material of both *C. laterale* and *C. carolinianum* have been undertaken to determine the relative position and behavior of the nuclei in development.

Results so far obtained do not support the probability suggested by Karling. The nucleus of both *C. laterale* (fig. 20) and *C. carolinianum* (fig. 26) remains in the extramatrical zoospore cyst during the development and maturation of the intramatrical rhizoidal system and apophysis. Prior to development of the zoosporangium, the nucleus within the zoospore cyst of *C. laterale* is ovoid, its widest diameter being 2.2 μ. In *C. carolinianum* it is similarly shaped at this stage but larger, its greatest diameter ranging from 2.4 to 2.6 μ. With the onset of zoosporangial development, however, the nucleus in both species enlarges. In *C. laterale* it measures approximately 3.7 μ (fig. 21, 22) and in *C. carolinianum* 4.5 to 4.9 μ (fig. 27). There may be a proportional relationship between increase in nuclear size and sporangial size, but until hundreds of nuclei have been measured, generalization is hardly warranted. The most that can be stated at present is that in both forms there is a measurable increase in the size of the primary nucleus as the zoosporangium begins to develop from the persistent cyst of the zoospore. While the incipient zoosporangia enlarge, the primary nuclei divide. Figure 23 of *C. laterale* shows a binucleate incipient sporangium with the nuclei in the prophases of the second division, while figure 29 shows a binucleate sporangium of *C. carolinianum* with the two nuclei in the metaphases of the second division. Metaphase nuclei of *C. laterale* are shown in figure 24. Nuclear division in both forms therefore is mitotic, intranuclear, and synchronous. In both species, prophase stages similar to those of *Cladochytrium replicatum* (Karling, 1937) and *Endochytrium operculatum* (Hillegas,

differentiated as in figure 5. ×3360.—Fig. 11. Later stage in the development of an intramatrical absorbing system initially differentiated as in figure 7. ×3360.—Fig. 12. Intramatrical absorbing system with extensive rhizoidal catenulations. ×2520.—Fig. 13. Development of the zoosporangium as a lateral outgrowth of the persistent zoospore cyst. ×2520.—Fig. 14. Zoosporangium with evenly granular contents and the operculum fully delimited. ×2520.—Fig. 15. Discharge of zoospores. ×2520.—Fig. 16–19. Resting spores developed intramatrically by encystment of the predominant primary apophysis. ×2520.

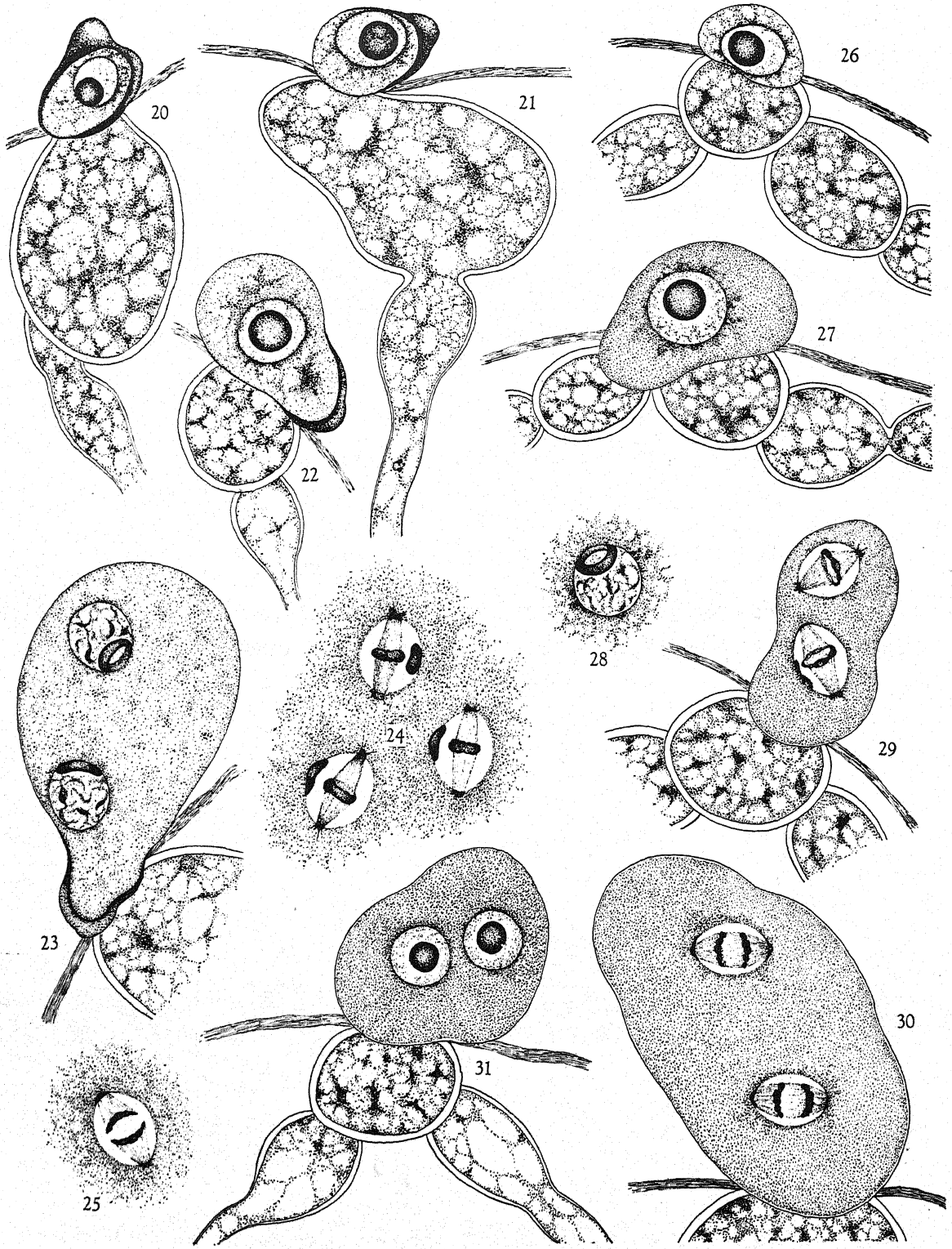


Fig. 20–31.—Fig. 20–22. Development of the zoosporangium and enlargement of the primary nucleus in *C. laterale.* ×5040.—Fig. 23. Prophases of the second nuclear division in *C. laterale.* ×5040.—Fig. 24. Metaphases of *C. laterale.* ×5040.—Fig. 25. Anaphase of *C. laterale.* ×5040.—Fig. 26, 27. Development of the zoosporangium and enlargement of the primary nucleus in *C. carolinianum.* ×5040.—Fig. 28. Prophase of *C. carolinianum.* ×5040.—Fig. 29. Metaphases of the second nuclear division in the zoosporangium of *C. carolinianum.* ×5040.—Fig. 30. Anaphases of the second nuclear division in *C. carolinianum.* ×5040.—Fig. 31. Binucleate incipient sporangium of *C. carolinianum.* ×5040.

1940) have been found. As in the species studied by Karling and Hillegas, the nucleole of *C. laterale* is very prominent at metaphase (fig. 24), but in *C. carolinianum* it is lacking or reduced (fig. 29). However, the number of division figures so far observed is small and further observations are essential before it can be said that the nucleole is lacking or is more reduced at the metaphase in *C. carolinianum* than in *C. laterale*. Individual separate chromosomes have not been recognized in the equatorial plate stage of *C. laterale* (fig. 24), but in *C. carolinianum* the separate chromosomes appear as irregular lumps in the equatorial ring and anaphase groups (fig. 29, 30). Fixation and staining may account entirely for this difference in appearance. Overstaining causes the rings of chromosomes to appear as a homogeneous mass (fig. 24), but optimum differentiation suggests that discrete entities compose the ring (fig. 29).

Unfortunately, no resting spores of either species have been available for cytological study. None developed in cultures of *C. carolinianum,* and the few that developed on two occasions in cultures of *C. laterale* were kept for study of their germination. The position of the nucleus in relation to the resting spore, and the germination of the latter therefore remain to be determined.

Relationships.—Inasmuch as the apophysis of *C. laterale* may be compound or single, this fungus shows affinities with *Catenochytridium carolinianum* and species of the genus *Chytridium*. Therefore, it is questionable whether or not *Catenochytridium* is sufficiently distinctive to merit generic rank. The nature of the apophysis and rhizoids by itself is a doubtful generic character, and inasmuch as *Catenochytridium* appears to be similar to the majority of *Chytridium* species in all other respects, there is but little reason for separating the genera, so far as they are now known. However, the genus *Chytridium* varies considerably and includes at present non-apophysate species (*C. sphaerocarpum,* Dangeard, 1890–91; *C. papillatum,* Sparrow, 1933a), as well as non-rhizoidal species (*C. inflatum,* Sparrow, 1933b; *C. Scherffelii,* Sparrow, 1936). In view of this, and the fact that the two species of *Catenochytridium* are incompletely known, it seems advisable to retain the genus *Catenochytridium* for the time being.

SUMMARY

Catenochytridium laterale occurs as a saprophyte on vegetable debris in soil collected near Sharon, Connecticut. In structure and development it shows affinities with both *Catenochytridium carolinianum* and certain species of *Chytridium*. Resting spores develop asexually, but their germination has not been observed. The relation of nuclei to the development of the zoosporangia of *C. laterale* and *C. carolinianum* is similar. In both species, the nucleus of the zoospore remains in the persistent extramatrical zoospore cyst, while the intramatrical absorbing system develops. When exogenous development of the zoosporangium begins, the nucleus enlarges before karyokinesis. Division is mitotic, intranuclear, and synchronous.

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VEGETATION OF THE COAST OF ECUADOR AND PERU AND ITS RELATION TO THE GALAPAGOS ISLANDS. I. GEOGRAPHICAL RELATIONS OF THE FLORA [1]

Henry K. Svenson

DARWIN'S VISIT in 1835 brought the Galapagos Islands to a prominence far greater than their size and economic conditions would warrant, due to the strange animal and plant life which up to that time had been relatively unchanged by human interference. Here Darwin, after observing the peculiar birds and giant tortoises, got his idea of evolution by geographic isolation, since many related species appeared not only to be endemic but also to be confined to individual islands of the archipelago. Upon naming the plant collections made by Darwin, Hooker found (1847a, p. 235), "a flora differing by upwards of one-half its species from that of the rest of the globe, a peculiarity shared by no other tract of land of equal size, excepting perhaps the Sandwich group." Stewart (1911), estimated 40.9 per cent of the plants to be endemic, but after my visit to these islands in 1930 as a member of the Astor Expedition, it became apparent that many of the supposedly endemic species were not at all different from plants of the arid coasts of Ecuador and Peru, which have only recently become adequately known. With the problem of origin of the Galapagos flora in view, I visited the coastal area of Ecuador and Peru from January to April in 1941, fortunately in one of those seasons when heavy rains fall in a region of normally sparse rainfall.

For travel funds I must thank the John Simon Guggenheim Memorial Foundation, and the kindly administration of the Secretary, Henry Allen Moe. At Guayaquil, I was indebted to Sr. Ayala, Dirección de Agricultura del Litoral, to Sr. Cordovez, and to Dr. Herman Parker (a friend to all visiting scientists); at Salinas, to the late Col. Walter K. Burgess of the United States Air Mission to Ecuador, to Mr. William Hunter (who made possible the exploration of the Chanduy Hills), and especially to Mr. Frederick Kenward, Director of the All-America Cable Station. At Talara, officials of the Imperial Oil Co. were most cooperative, and I spent several days with Oscar Haught, who knows better than any one else the flora of that region.

Botanists at Harvard University and at the United States National Herbarium have assisted in identification. Dr. Robert Cushman Murphy, of the American Museum of Natural History and Dr. H. E. Stork have been helpful in many ways. Finally, my thanks go to the late Dr. C. Stuart Gager, Director of the Brooklyn Botanic Garden, for his continued interest in the project.

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GEOGRAPHY

The three areas under discussion form an equilateral triangle, with its base in Ecuador and Peru and its apex to the west in the Galapagos Islands. From the Ecuadorean-Colombian boundary (1° 30′ N) south to Talara, in northwest Peru, it is approximately 500 miles, and the Galapagos Islands lie the same distance from the Ecuadorean coast. Western Ecuador consists of a narrow strip of comparatively low mountains, forests and savannas. Guayaquil lies about 75 miles east of the open waters of the Pacific, at a latitude where the South American continent bulges to the westward, the greatest extension being at Punta Pariñas (approx. 81° 20′ W) near Talara. Physiographically the terrain is much the same on both sides of the Gulf of Guayaquil except for increasing aridity southward. The Galapagos Islands comprise nine larger islands and many small ones (fig. 1). From north to south they extend slightly over two hundred miles, but the great bulk of land surface lies between the equator and 1° S. According to Wolf (1892, p. 12), the land area is 7430 sq. km. (2869 sq. mi.). Albemarle, the largest, extends 75 miles in a north-south direction. They are volcanic islands with lava flows and craters of varying age. On Albemarle the volcanic peaks reach 4700 ft. and on Narborough, 3720 ft.[2] Some of the smaller islands to the northward are separated from the main group by rather deep water.

GALAPAGOS ISLANDS.—First mention of the geology seems to be the account of volcanoes which Thornton (Philosophy of Botany 1: 137. 1810) quotes from the versatile Robert Hooke (1635–1703): "Nor do I conceive that all those clusters were all thrown up at once, as the *Grecian* islands in the *Archipelago,* the *East-Indian* Islands in that part called the *South Sea,* . . . the isles of Cape Verd, Canaries, Orcades, etc., also the *Gallopegas* and others in the *Pacific Sea,* or *Mar del Zur;* but rather that some were made in one age, and some in other ages of the world." In general the Galapagos Islands have been considered as pelagic islands of volcanic origin, and the plants and animals are thought to have been introduced sporadically from the mainland. Wolf, the Ecuadorean geologist, was in the Galapagos Islands from August to November, 1875, and from May to July, 1878. He notes (p. 471) that the islands are exclusively volcanic, and that they have not been formed by disruption of a more extensive terrain, or by separation from the South American continent, or by a rise in the

[2] Gt. Brit. Foreign Off. Hist. Section no. 140. 1920. This appears to be the best general and historical account of the Galapagos Islands.

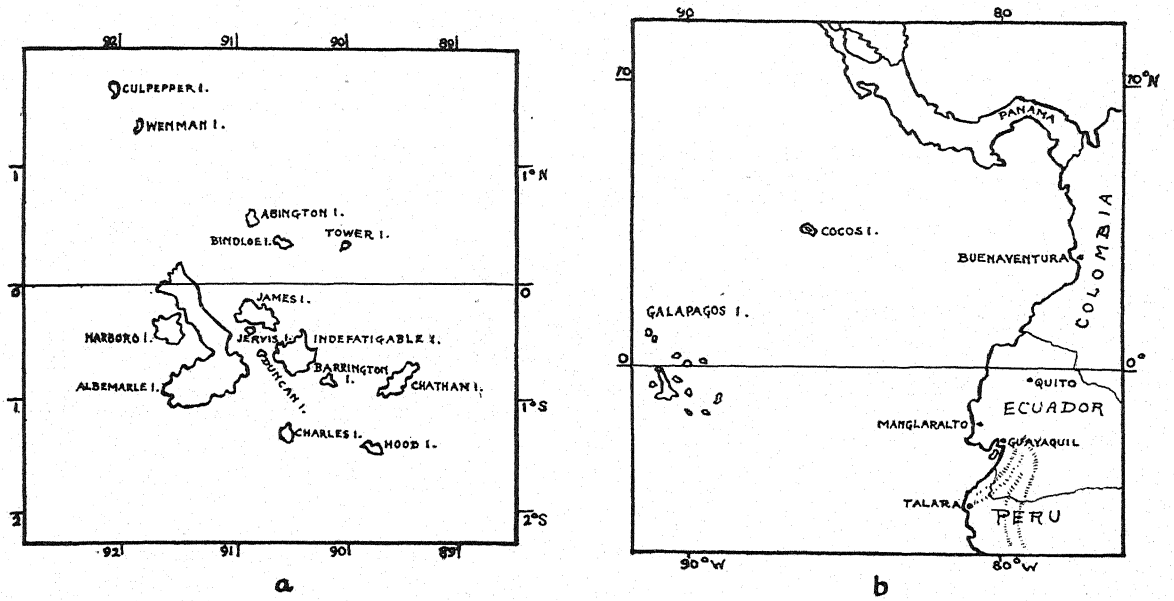


Fig. 1. (a) Galapagos Islands, from Wallace, *Island Life*, p. 267, (b) map showing relations of Galapagos and Cocos Islands to mainland, from Wallace, *Island Life* p. 266.

ocean floor, but simply by successive accumulation of eruptive material which finally has arrived at sea level. Almost all the islands (p. 472) show evidence of increase from a central point, usually a principal crater, and by outpouring of lava have extended their periphery in many directions. Many lateral and secondary craters were formed at the side of the central and principal crater, as is clearly shown on Indefatigable Island. Further (p. 473), the formation of the Archipelago has no relation to that of the Andes of Quito, which are of andesitic material, while the Galapagos Islands are basaltic; the most remarkable phenomenon being the presence of old light-colored projecting crater rims of volcanic tuff, partly overflowed by dark basaltic lavas. After examining Baur's reports, Wolf (p. 656) believed that the subsidence theory of Baur increased rather than decreased the difficulties of the origin and distribution of species in the Galapagos Islands.[3] On the other hand, the islands are known to occupy a plateau in the Pacific Ocean extending northeastward to Cocos Island. According to Rehder (1940, p. 114), the Galapagos Islands were probably separated from the coast by downfaultings and "the island mass gradually subsided, leaving above the surface of the water only the mountain tops to which the animal life gradually withdrew as the land subsided. Later the islands rose again, as is evidenced by the presence of fossilliferous beds on Albemarle, Indefatigable and Seymour Islands, of which some at least are of Pliocene age, and which are both underlain and overlain by lava deposits."[4]

Howell (1941, p. 239) stresses the predominantly basaltic nature of the islands, the lava-types being North American, rather than the andesitic lavas of South America. Schuchert (1935, p. 629), however, states that the Western Cordillera [of Colombia] consists mainly of basic eruptives (diorite, diabase, gabbro, and serpentine) and older unfossiliferous slates and phyllites. The rapid growth of the new volcano "Paricutin" in Mexico shows how quickly such structures may rise. Whether the Galapagos Islands are closely allied to the recent volcanoes of southern Mexico and Central America (cf. Schuchert, p. 11 and fig. 5) is not known.

Beginning with Baur (1891–1897) there has been much discussion as to whether the Galapagos Islands represent emerged portions of a larger land mass, perhaps connected with the North American or South American continent. With lack of stratigraphic correlation, an answer has taken the form of comparison of the floras of the various islands. Baur divided plant and animal populations of oceanic islands into two sorts: those of obviously sporadic nature in which species have no local coherences of inter-relationship and in which plants seem dependent on seeds borne by winds, waves, and birds ("disharmonic"); and those, such as the Galapagos, where groups of islands have attained

[3] "Ademas me parece que con su hipotesis (de una separacion y submersion sucesiva de las islas) la explicacion del origen y de la distribucion de las especies, admitiendo con el un desarrollo natural segun la teoria de descension, no se facilita, y talvez se dificulta mas la de ciertos fenomenos."

[4] This account of fossiliferous beds probably came from Stewart (1911, p. 238), who mentions deposits of marine fossils elevated a few feet above sea level on these three islands, the old beach at Albemarle being 60 ft. above present sea level. He found no evidence of a general elevation outside these localities. Dall (Geol. Mag. 61: 428–429. 1924) believes this deposit to be late Pliocene, therefore showing that the islands have been in existence and apparently not much smaller since that time.

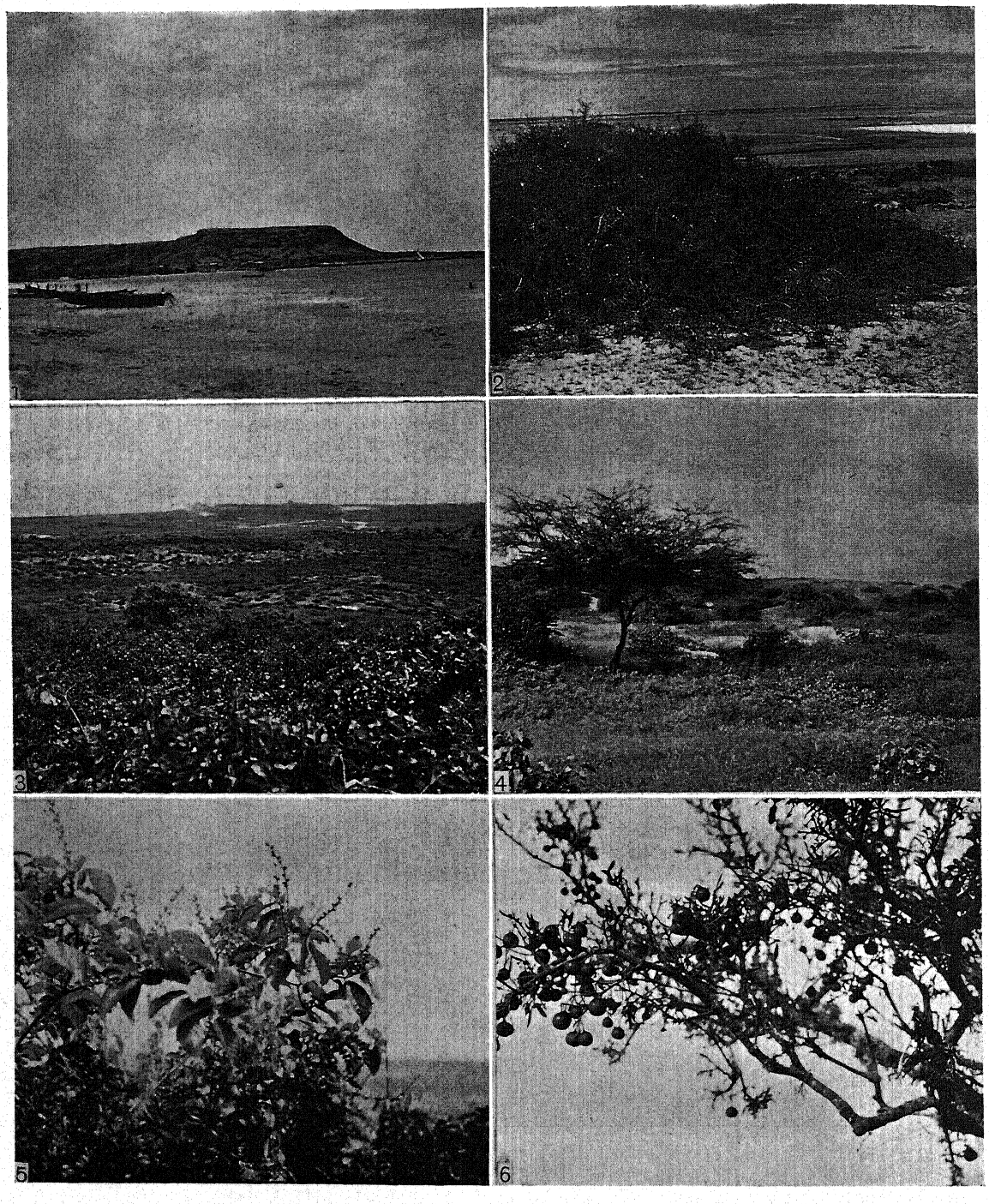
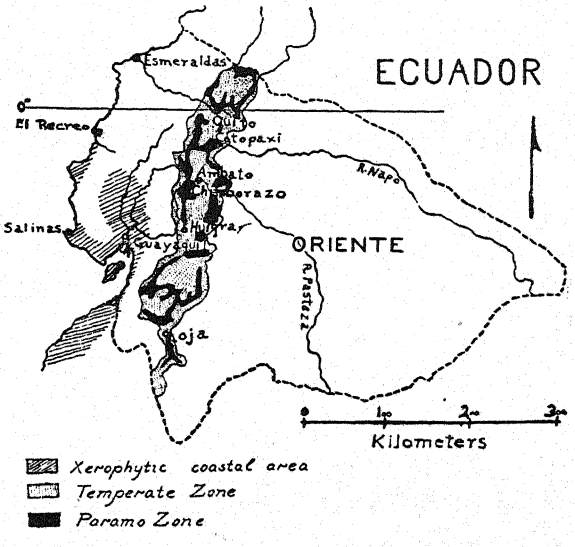


Plate 1. Fig. 1. Ecuador. Puntilla, Salinas.—Fig. 2. Ecuador. *Scutia spicata*.—Fig. 3. Ecuador. N.W. from Santa Elena to Punta Centinela.—Fig. 4. Ecuador. Salinas, *Prosopis inermis*.—Fig. 5. Ecuador. *Croton rivinaefolius*.—Fig. 6. Ecuador. *Jacquinia pubescens* (in fruit).

an internal harmony ("harmonic"), due to descent of the plants and animals from a limited number of original importations, which were at the start miscellaneous and disharmonic. From island-to-island distribution of genera, he concluded that the Galapagos represented an area of subsidence, originally a single land-mass attached to the continent. As Baur saw the situation, the species (or forms of species) are frequently confined to a single island, and the species are more closely related to those of other islands in the group than to any continental ally.

Robinson (1902, pp. 253–260) concluded that distribution of species on the Galapagos Islands was easier to account for by the older idea of emergence with reasoning as follows: Suppose seeds of a certain species arrived from the mainland only two or three times in a century. It is well known that introduced plants can quickly overrun territory. New races, showing slight variation in early seedlings, would be formed in the altered environment either by direct influence or natural selection. Succeeding arrivals would not swamp out the original lot, for the first arrivals would hold possession. Such conditions would occur on an archipelago either emergent or subsident. Infrequent success of seeds would be due largely to the sterile, inhospitable shores. Differences between the Galapagos and the continental floras might perhaps be due to extinction of ancestral forms on the continent, and the relative similarity of various florulae of the islands, as compared with that of the continent, to less competition than in the larger flora of the mainland. "It is better to admit a nullity of botanical evidence in regard to this interesting question than to attribute to the 'harmonic' flora of these islands an historic meaning which it may not possess." The distant islands, often separated by relatively deep channels, often show more floral similarity than the nearer ones. This should not be the case if subsidence had taken place. And finally: "These anomalies in the different florulae must find their explanation in peculiarities of climate and soil, together with an element of chance—arising partly from imperfect exploration, and partly from the accidents of seed-dispersal." After Stewart's explorations had provided further data, Kroeber (1916) analyzed botanical relationships and concluded that the statistical method employed previously had little value, and that (p. 208) "in general the number of species common to any two islands depends not so much on their location relative to each other, nor on their altitude, rainfall, or position in certain winds or currents, nor on any as yet unknown or mysterious cause, but on the mere wealth and variety of their plant lives. This is the all-important factor, beside which every other is comparatively insignificant. Where this element is considered, the internal floral relations of the Galapagos are substantially accounted for; when it is disregarded, they become unintelligible." It seems that the fundamental error lay in the comparison of islands, without reference to individual size, climate or soil; secondly, species, varieties and forms were all treated as of equal value.

Howell (1941), studying the geographical distribution of *Scalesia* (Compositae), decided that there was no conflict with a theory of an emerged region, where the islands now are, and a continental mass to the northward or eastward or with accidental introduction of the genus *Scalesia* on a Galapageian island of oceanic origin. He states (p. 238) that the progenitor of Scalesia may not have been highly specialized, but this assumption is even more theoretical than the presence of a large Galapagos land mass with continental connections. Acosta Solis (1937, p. 11), after a visit to the islands, corroborated Wolf's views, and Howell feels that "from insular studies in the several branches of geology will eventually come the answer that can only be theoretically approached in botany and zoology" (1941, p. 239).



THE VEGETATION OF ECUADOR

Fig. 2. Vegetative zones in Ecuador showing especially the xerophytic area. Courtesy of *Chronica Botanica* (1943).

SOUTHWESTERN ECUADOR.—By Eggers (1894) the area is divided into an inner and an outer coastal plain. The former, occupying the region at the head of the Gulf of Guayaquil, is covered by vast mangrove swamps, by extensive savannas flooded during the wet season, and by plantations of cocoa and tropical fruits. It is separated from the outer coastal plain by a broken range of north-to-south hills, about 1000 ft. high, the Cerro de la Estancia and the Cerros de Chanduy (cf. Pl. 4, fig. 4).

The outer coastal plain has been well studied by the petroleum geologist Sheppard (1930 and 1937). Oil is obtained in the flat country near Ancon, and the Santa Elena peninsula is underlaid by a stratum of asphalt. Large bones of Pleistocene mammals, somewhat similar to those of the La Brea deposits of California, are found near the village of Muy. The flat area is covered by *tablazos,* upraised sea floors and beaches of Quaternary age. The extensive salt deposits worked at Salinas (Sheppard, 1932) were formed in lagoons abandoned by the sea, as a result of the uplift which has been in operation from the close of the Quaternary period. Low residual Tertiary hills of chert, shale, conglomerate, or igneous intrusions, rise up here and there from the flat country, and have resisted the rapid erosion which occurs in occasional years of

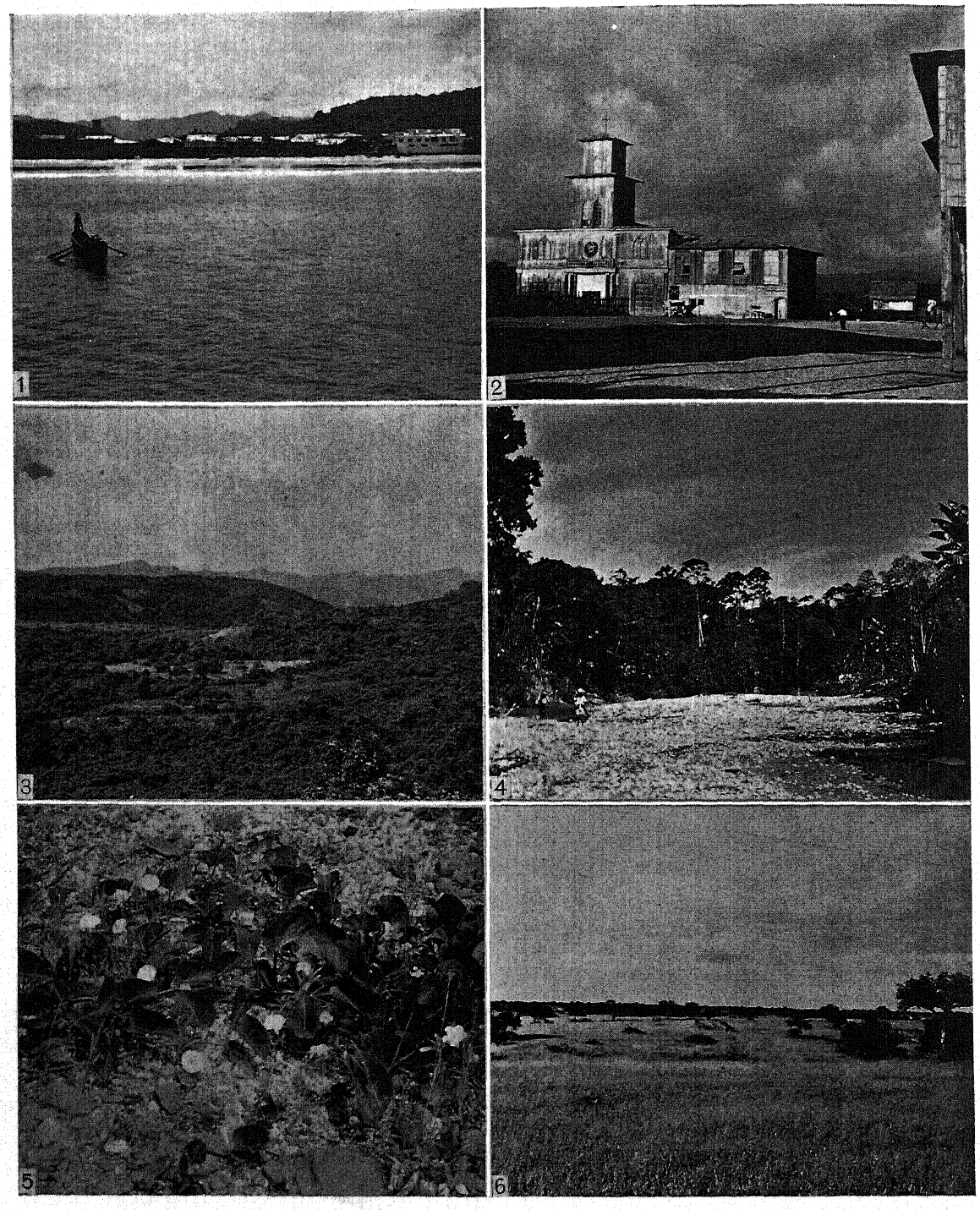


Plate 2. Fig. 1. Ecuador. Manglaralto.—Fig. 2. Ecuador. Manglaralto.—Fig. 3. Ecuador. View east from Manglaralto.—Fig. 4. Ecuador. River, Manglaralto.—Fig. 5. Peru. *Ipomoea crassifolia*.—Fig. 6. Ecuador. Savannas e. of Chanduy, *Jacquinia* trees.

heavy rainfall. The westernmost is the flat-topped point of Santa Elena which, according to Wolf (p. 21), is 129 m. [423 ft.] high (see fig. 3 and pl. 1, fig. 1). It extends out into the Pacific as the westernmost point of Ecuador, and appears as an island when seen from a distance.[5] The flat-topped

[5] Dampier (p. 133) landed at Santa Elena in 1684. "The Point is pretty high, flat, and even at top, overgrown with many great *Thistles* (cacti), but no sort of Tree. The Land about it is low, sandy and barren, there are no trees nor grass growing near it; neither do the *Indians*

ridge north of Santa Elena, the Cerro de Ballenita, is placed at right angles to prevailing southwest winds, which Sheppard (1930, p. 447) believes have played a large part in sculpture of land surface into topographical ridges tending northwest to southeast. A similar ridge extends from Punta Centinela (cf. pl. 1, fig. 3, and pl. 4, fig. 1), a few miles to the northward, to the village of San Vicente. The vertical seaward slope of Punta Centinela is marked by fantastic foldings and faultings of strata, showing the effect of crustal disturbances.

North of Punta Ayangue the country becomes very rough, due to the proximity of the Cordillera de Colonche to the shore (pl. 2, fig. 1, 3). This range of hills, rising to at least 2500 ft., is nearly always shrouded in mist and clouds due to condensation of moisture of winds from the southwest. To the north the cone-shaped old volcanic peak of Monte Cristi rises higher, and the Cerro de Isera is a landmark at the southern boundary of the Cordillera de Colonche. At its southern extremity this range bends sharply to the eastward, forming the low Chongon Hills which terminate at Guayaquil. To the northward, rocks of igneous origin form the promontories of Salango and San Lorenzo. On the western declivity of the range small streams cut deep gorges through massive Tertiary sandstones and conglomerates. These streams, Sheppard (1937, p. 450) believes, formed the eastern tributaries of a larger river which has been drowned, the western tributaries of which are to be traced on La Plata Island,

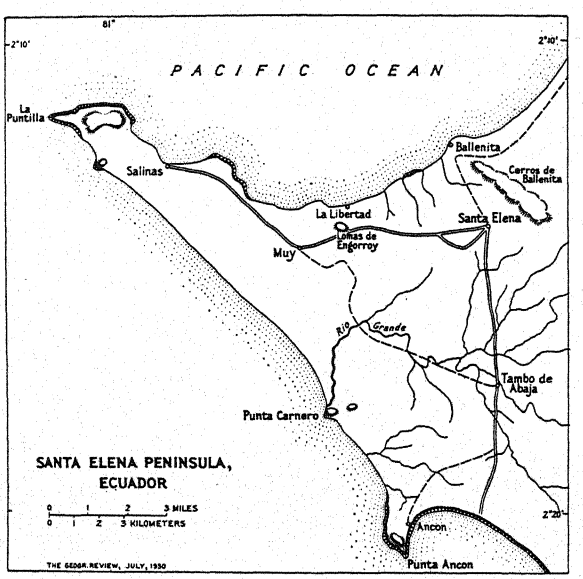


Fig. 3. Santa Elena Peninsula, the westernmost point of Ecuador. Sheppard (1930). Courtesy of the *Geographical Review* published by the American Geographical Society of New York.

produce any Fruit, Grain, or Plant, but Watermelons only, which are large and very sweet. There is no fresh water at this place, nor near it, therefore the Inhabitants are obliged to fetch all their water from the River *Colonche,* which is at the bottom of the Bay, about 4 leagues from it. Not far from the town on the Bay, close by the Sea, about 5 paces from high-water mark, there is a sort of *bituminous* matter boils out of a little hole in the Earth; it is like *Tar;* the Spaniards call it *Algatrane.* By much boiling it becomes hard like Pitch. It is frequently used by the *Spaniards* instead of Pitch; and the *Indians* that inhabit here save it in jars."

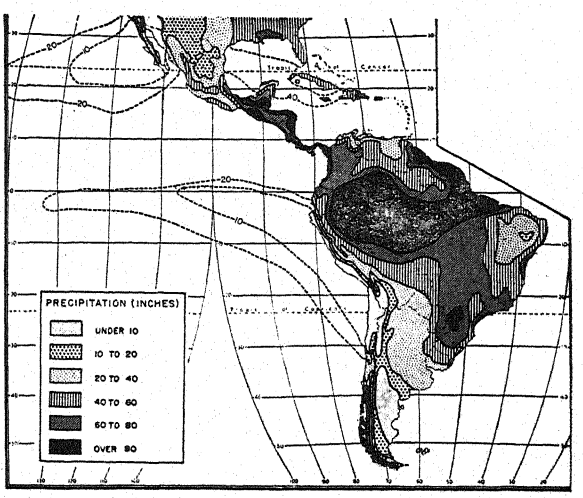


Fig. 4. Annual precipitation map showing the 10–20 inch zone in which lie the Galapagos Islands, the southwest coast of Ecuador, and the inner coastal area of Peru. W. Reed in *Climate and Man,* U. S. Yearbook of Agriculture, 1941. Fig. 3.

about fifteen miles from the mainland. This island—Dampier's headquarters while a pirate—consists of Tertiary shales and cherts, and of fine-grained volcanic post-Tertiary rocks. It was separated from the mainland in recent times (Sheppard, 1927, p. 484): "The close of the Tertiary period was marked by a gradual tectonic uplift which involved the littoral, or coast-belt, of both Ecuador and Peru, this probably being the consequence of a major movement initiated from the axis of the main Andean upthrust. The coastal region of Ecuador had no doubt a much greater westerly extension than obtains at the present time; it must have persisted beyond the present Isle de la Plata and probably included the area now known as the Gulf of Guayaquil. Hence it formed a northerly continuation of the Peruvian littoral" (p. 484).

Northwest Peru.—Bosworth's (1922) work on the desert region of northwestern Peru formed the geological background upon which Weberbauer (1929) treated the vegetative zones (fig. 11). Extending northeast-southwest and rising abruptly from the plain at a distance of 12–16 miles from the coast is the westernmost spur of the Andes, the Amotape Mountains. They are composed mostly of shales, calcareous slates and granite intrusions of Palaeozoic age. Cerro Prieto, part of the western escarpment (pl. 5, fig. 6), reaches 3000 ft.; a few miles further inland Cerro Viento rises to 5000 ft. Resting unconformably against the base of the mountains are the Tertiary and Quaternary deposits which extend to the Pacific Ocean. These show in a spec-

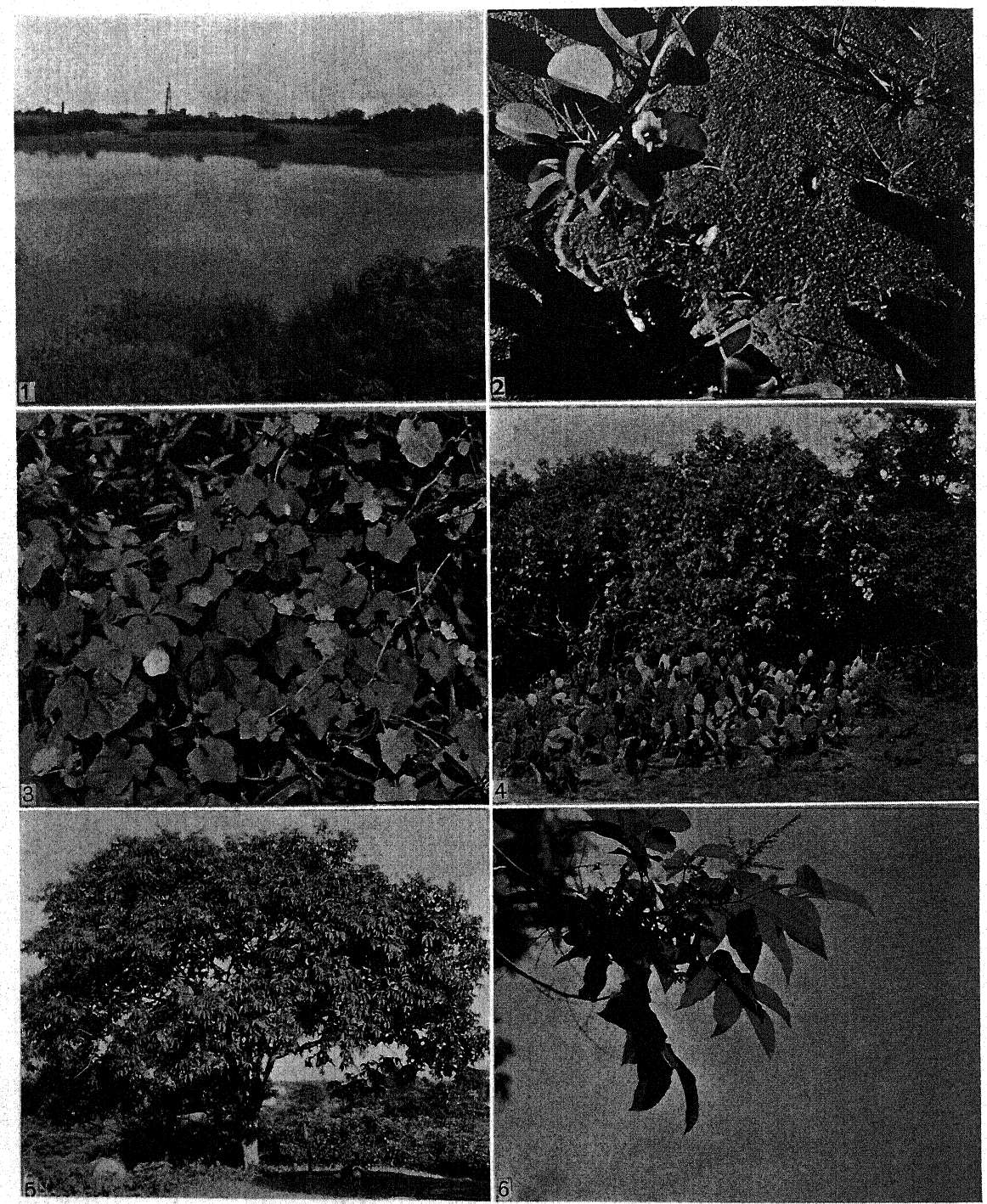


Plate 3. Fig. 1. Ecuador. Pool near Salinas.—Fig. 2. Ecuador. *Ipomoea Pes-caprae.*—Fig. 3. Peru. La Brea, *Ipomoea aegyptia* and *Luffa Astori.*—Fig. 4. Ecuador. Punta Ayangue, Opuntia sp.—Fig. 5. Ecuador. Near Engabao, *Loxopterygium Huasango.*—Fig. 6. *Loxopterygium Huasango* (branch).

tacular manner the work of erosion which the various *tablazos,* or raised sea-beaches, have undergone, both by winds and by the heavy rains which fall at infrequent intervals. At the coast are dunes and saline plains of recent age, and at Negritos (pl. 5, fig. 1) soft tertiary sandstones and shales come down to the shore. These rocks are overlaid by the Talara Tablazo (Quaternary) with a thickness of 8–25 ft. and an altitude of 150–300 ft. A second and much higher tablazo, the Mancora, with an altitude of

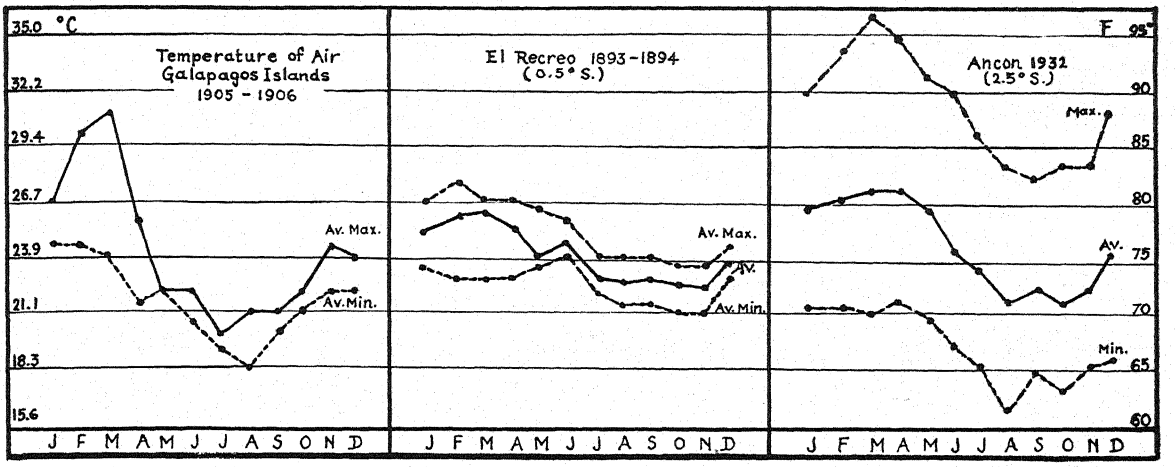


Fig. 5. Air temperatures in the Galapagos Islands and the coast of Ecuador. Galapagos data from Stewart (1911), based on comparatively few measurements; El Recreo, from Eggers (1894); Ancon, from Sheppard (1933—Courtesy of the *Geographical Review* published by the American Geographical Society of New York). representing the unusually warm and rainy year of 1932.

200–1200 ft. and a thickness of 75–250 ft., forms a prominent flat skyline northward from the base of the Amotape Mountains. Extensively faulted Tertiary beds reach an aggregate of 20,000 ft. or more. Due to faulting, erosion of the tablazos, and the building up of breccia fans at the base of the mountains, the topography is that of the bad lands (see pl. 5, fig. 7). The streams cut deep quebradas in the mountain rocks; in the tablazo area, these gorges flatten out into valleys, bordered by prominent terraces of various levels, formed by the deluges that come at intervals of from seven to thirty years. During my stay at Talara, the usually dry Pariñas River was at flood stage, with people and vehicles encamped on both sides waiting for the waters to subside. Quebrada Ancha lies close to the village of La Brea, where petroleum pits were worked by Indians and Spaniards as a source of pitch. Quebrada Mogollon lies a few miles to the north. The Chira River, the southern boundary of the Amotape region, has its sources high in the Andes, and its irrigated margins provide the only cultivated land of any great extent until the Ecuadorean boundary is reached northward at the Tumbez River.

The coastal plains of Peru are composed of Tertiary and post-Tertiary sediments and lava flows. In Tertiary time they were more extensive. The northern division begins at Tumbez and extends southward (cf. Marsters, 1912, pp. 228–238 and map opp. p. 227) reaching its greatest breadth in the region of the Sechura Desert at about 6° S. Then it narrows rapidly and ends at Salaverry. From this region to Lima the foothills of the Cordillera approach the coast and the typical coastal plain is not found again until the region of Cañete and Chincha (about 14° S) is reached. The plain broadens out in the Pisco-Ica region and disappears again in the vicinity of Lomas (16°); then as a series of raised plains back of the coastal ridges it extends to the Chilean border. It is not yet known whether the *Alternantheras* [see Catalogue of Plants, no. 86–95] common to the Galapagos Islands and these southern coastal areas have their distribution limited chiefly to the coastal plains.

CLIMATE

Galapagos Islands.—Both temperature and rainfall show great variability in the Galapagos Islands. Even explorers have not been willing to remain long enough in any one place to obtain satisfactory records of temperature and rainfall. Nor is it probable that such records would do more than emphasize the great variation locally and from year to year. The best available long-range records of temperature are those of the Expedition of the California Academy of Sciences in 1905–06 (Stewart, 1911, p. 224–227) which I have arranged in graphic form to compare with those obtained by Eggers at El Recreo and Ancon on the coast of Ecuador (fig. 5). These readings, obtained from place to place during the cruise of the "Academy," show that March is hottest, with noon air temperatures uniformly about 88°F. (early morning temperatures 70–78°F.), and lowest in August (68° and 64° respectively). A record of winds is given by Slevin (1931) in the "Log of the 'Academy.'"

The Galapagos Islands lie within the influence of the Humboldt Current which sweeps up the coast of Peru, and have a reputation of being cool and agreeable. I found it quite otherwise during the first two weeks in April, 1930, and Chapin, on a return visit in March, 1935, found the same intolerable heat (1936, p. 36): "Some of my friends, even after visiting the Galapagos, describe them as cool and comfortable, despite their equatorial location. I am a violent dissenter, in so far as the months of March and April are concerned, when my visits occurred. Other seasons may be different."

Wolf (p. 477), in the Islands during cooler seasons, kept temperature records on Charles and Chat-

ham. He states that 22°C. [71°F.] is the average temperature up to 100 m. altitude, but days are hotter and nights cooler than this average. On Charles at 133 m. elevation for many days the temperature averaged only 20°C., and higher up at the hacienda (277 m.) between 18° and 19°. On Chatham, at 288 m., for ten days the average was 19°C.; between 300 and 400 m., it was 18°; and on the *Cerro de San Joaquin,* the highest point on Chatham Island, it was only 14° [57°F.] at noon, with dense fog and a strong wind blowing from the southeast. The dry zone extends in general to 220 m., and therefore includes most of the land area of the archipelago, since only the upper parts of the larger islands are in the humid zone. Rain falls on the islands at the same season as in Ecuador (from February to June), but is very irregular, and there are years in which it fails completely. At this season rains moisten the arid lowlands, and the vegetation receives the necessary moisture for existence. In the high zone (p. 479) it rains more in summer than in winter. During Wolf's stay, from August to November, the mountains of all the islands were almost continuously enveloped in clouds and fog; in the upper part of Chatham he did not see the sun for ten days and suffered from continuous rain, although in the northern half of the island, in the low zone, not a drop of rain fell. The wind blows almost always from the southeast, and since it carries the vapors which condense on the high mountains, the humid zone is 40–70 m. lower on the windward side of the mountains than on the opposite side.

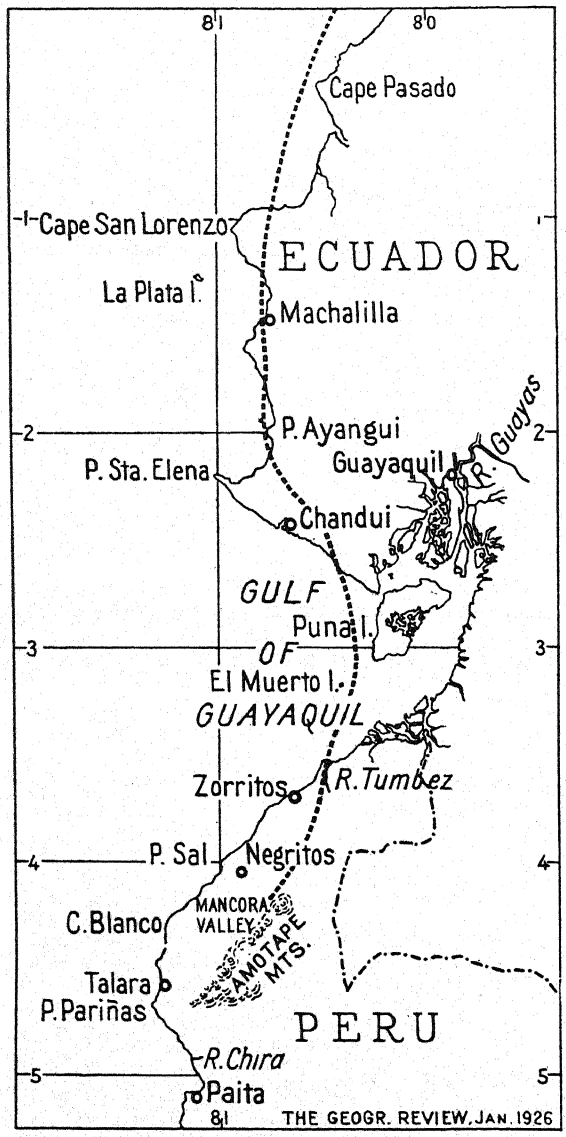


Fig. 6. Rainfall distribution in western Ecuador and Peru. West of this line the annual rainfall is scanty or entirely lacking. Murphy (1926). Courtesy of the *Geographical Review* published by the American Geographical Society of New York.

During Stewart's stay (1905–06) there were no heavy rains at sea-level, but erosion showed that they must have occurred from time to time. The season of 1906 was exceptionally dry, with no rain falling on Chatham Island from March till July. As a result, vegetation was parched even at the summit (2100 ft.), and Stewart (1911, p. 220) noted similar conditions on Charles during May and June. On Indefatigable, 1930 was the dryest year since 1926 (according to one of the colonists at the plantation Fortuna), when water had to be brought down in kegs from the summit of the mountain. On the other hand, so much water swept down in 1929 that much of the soil in the plantation was washed out. As Stewart states: "The places where precipitation is great enough to support a mesophytic vegetation are mostly confined to the middle and upper parts of the islands. The moisture here is derived from the fog banks which strike the windward sides of the mountains at various elevations, whereupon the fog is thrown down as fine mist and sometimes as rain. These fog banks, however, do not always extend to the tops of the mountains, as these are often clear while the region a few hundred feet below may be entirely enveloped in fog. The soil at the tops of the mountains is sometimes dusty, while a little below the top it may be very moist or even muddy. From February until June inclusive there is much less fog in the upper regions than during the remainder of the year. During these four or five months the tops of the mountains may be entirely clear for several days at a time. The prevailing winds (p. 230) blow from the southeast, east-southeast, and south-southeast, and are the regular trade winds of this part of the Pacific Ocean. They blow quite regularly from June to January, but during the remainder of the year are very uncertain, and the waters surrounding the islands are subject to long periods of calm. . . . It is very seldom that the winds come from a northerly direction, and when they do they are usually of short duration. Storms are very rare, but short squalls sometimes occurred several times a day at Tagus Cove on Albemarle Island dur-

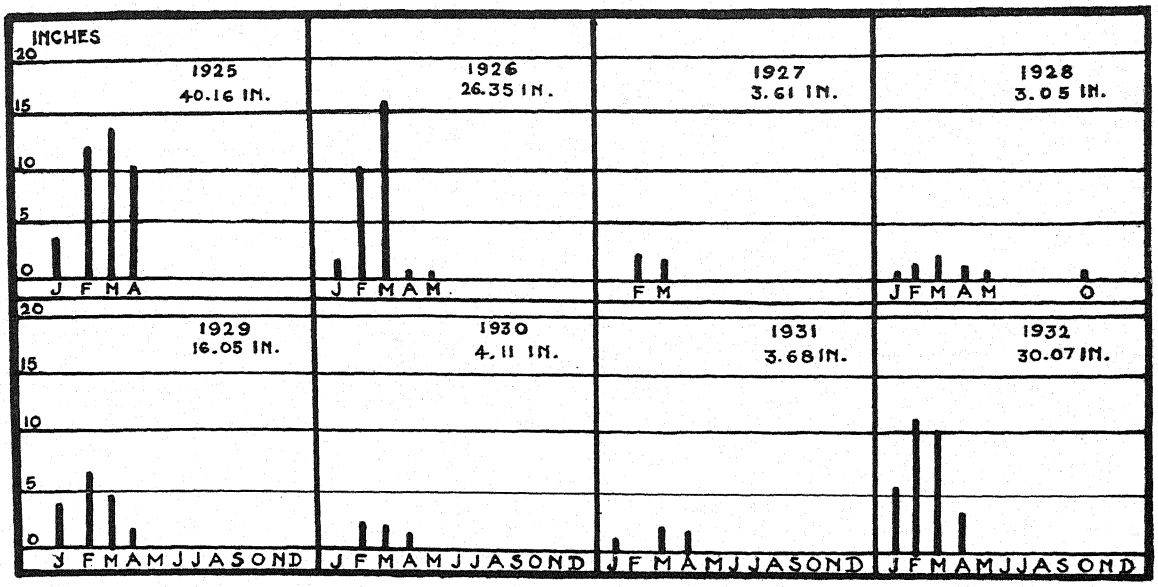


Fig. 7. Annual rainfall at Ancon (2° 20′ S.) on the coast of Ecuador (Sheppard, 1930, fig. 2; 1933, fig. 1). Courtesy of the *Geographical Review* published by the American Geographical Society of New York.

ing the months of March and April." The difficulty of such a climate for the human race is well expressed by Cutting (1940, p. 285): "The summit of the mountain looked down on a group of abandoned shacks, on a little graveyard, on the iron tank that still caught the rain and gave it back to the air—a symbol of futility."

Western Ecuador.—"I suppose there can be no region of the globe where there are so many climatic anomalies as interesting to the meteorologist." (Guppy, 1906, chap. xxxii: "The Coast of Ecuador.") Southwestern Ecuador represents the transition area where the normal November-April belt of tropical rainfall meets the arid and desert coast which is under the influence of the cold Peruvian or Humboldt Current.[6] This transition zone is a comparatively narrow strip extending in a northwest-southeast direction from the equator at Cape Pasado on the Ecuadorean coast to the vicinity of Guayaquil, and into the departments of Tumbez and Piura in Peru. It lies at right angles to the prevailing southwest winds which blow across the cold Humboldt current. In the same transition zone lie the Galapagos Islands. Wolf (1892, p. 385–388) understood the climatic situation well: "Except for fogs and garuas during the southern *invierno*[7] the wind vapor from the ocean is condensed only in contact with cool bodies, such as the littoral mountains and the higher Cordillera. In Peru, until high altitudes are reached, the mainland temperature is higher than that of the ocean. During the invierno, the solar heat diminishes; light garuas occur on the Peruvian coast, and heavier ones on the slopes of the Cordillera, but only enough for a verdure of grasses and herbs [i.e., the Loma formation] which soon disappear."

Murphy's map (1926) of western Ecuador and Peru is reproduced (fig. 6). It shows roughly the dividing line east of which rain falls annually, and west of which rain is known only at intervals of years. The more elaborate map by Troll (1930, fig. 343) draws the line through Puná Island, defines the inner limits of garuas, and provides a chart of the vegetation (certainly not correct as regards the isolated rainforests along the coast, which Wolf correctly delineates). I have assembled records of rainfall from various localities, showing variability of rainfall in this climate (fig. 7); these should be compared with Shreve's (1944) from northern Mexico. The 7-year cycle of heavy rains west of the line was broken in 1941 (with a 16-inch rainfall), whereas 50 inches had already fallen in 1939. During 1941, the heaviest rainfalls were on Jan. 24 (3.3 in.), Jan. 29 (2.25 in.), Feb. 7 (1.62 in.), Mar. 5, 1.0 in.), Mar. 7 (2.06 in.) and Mar. 27 (1.1 in.). In addition to supplying these records, Mr. Kenward tells me that each of the years 1925, 1932, and 1939, had between 30 and 50 inches of rain, and that intervening years have been dry—with half an inch to eight inches of rain. During the rainy season of 1941 there was a trace of rain nearly every day. At Ancon, a few miles away, the rainfall was much heavier, and it turned the black loam eastward to Guayaquil into an impenetrable morass swarming with mosquitoes. Heavy rains were accompanied by thunder and lightning, and violent gusts of wind from the north

[6] The best general account of these climatic conditions of the West Coast, with fine diagrammatic illustrations, appears in Robert Cushman Murphy's account, "To the Chocó in the schooner 'Askoy'" (Natural History, May, 1944).

[7] This is the opposite of the Ecuadorean "invierno." As Knoch (1930, p. G117) points out, the Ecuadorean summer and winter (*verano* and *invierno*) do not conform with the astronomical seasons, or with the usage in the countries to the southward. In Ecuador, summer is the cool dry season, and winter is the warm and rainy period.

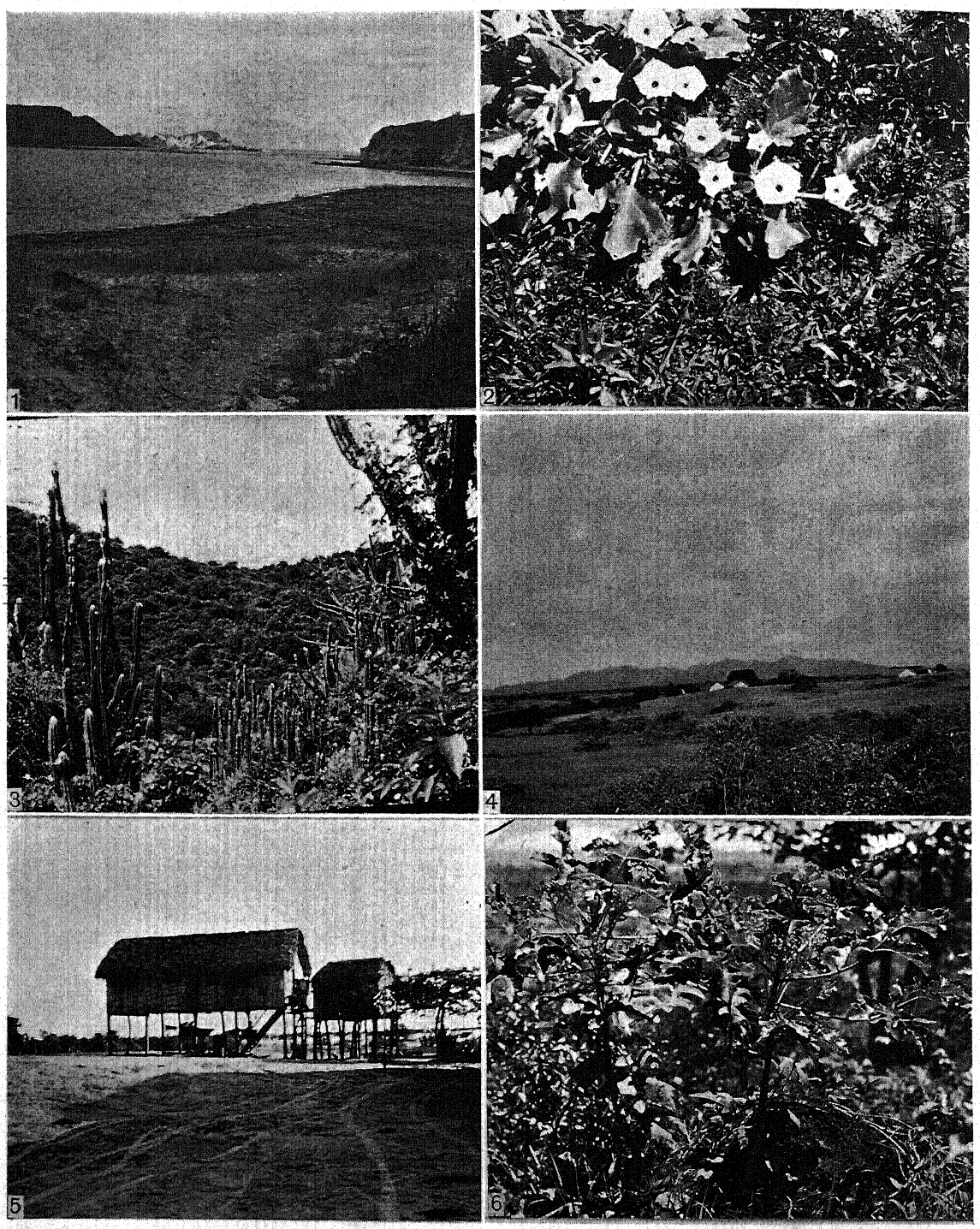


Plate 4. Fig. 1. Ecuador. Punta Centinela.—Fig. 2. Ecuador. *Cacabus prostratus*.—Fig. 3. Ecuador. Summit of Chanduy Hills.—Fig. 4. Ecuador. Savannas e. of Chanduy.—Fig. 5. Ecuador. Engabao, s. of Ancon.—Fig. 6. Ecuador. Santa Elena, *Carica paniculata*.

of such force as to tear the thatch from houses. Spruce (1908, p. 317) spent the rainy season of 1862 at Chanduy[8] some thirty miles down the coast:

[8] This town (pronounced Chan-dóo-y) has deteriorated a good deal since it is no longer a trading port. Ulloa (1760, p. 160) notes that the cool winds terminating the rainy period at Guayaquil were called the "chandui" since they blew from the direction of the Chanduy Mountains. His expedition, which included La Condamine and the botanist, Joseph Jussieu, arrived from Panama on March 9, 1736, to anchor in Manta Bay. Condamine

"Here it scarcely ever rains, beyond a slight drizzle in the morning, occasionally—the same as at Lima —and throughout the year 1861 there was but one day of heavy rain. This present year, however, we have had a real rainy season that began in February and lasted through most of March. It has been the first rainy season since 1845." Guppy (1906, p. 496) received reports from Santa Elena in 1904, saying that the rainfall for ten years was about two showers a year; also that the last good rainy season was in 1891, since which time there appeared to be a marked falling off in the rainfall.

Murphy (1926) was in Peru and Ecuador from December to March, and describes in detail the destructiveness of the rains of 1925 in the vicinity of Talara and Piura. During the month which he spent on the coast of Ecuador, only one entirely rainless day was encountered (1925, p. 447) and one rainless night: "The peninsula [Point Santa Elena] was green for the first time since 1919. . . . The middle land of the peninsula became a great morass; grass and shrubs sprang up everywhere from what had been bare ground; verdant lines worked down the seams of cliffs facing the sea. The captain of the steamer, which came close in shore while rounding Santa Elena on the route to Guayaquil, remarked that but for confidence in his own navigation, supported by his recognition of the wooden lighthouse, he would have believed himself in another part of the world. 'The lighthouse is the same,' he said, 'but the country is new.' " Perhaps because of remains of abandoned villages which are found in the arid part of northern Peru—which Bosworth considers to be futile attempts at settlement during comparatively rainy periods—the idea grew that the coasts of Peru and of Ecuador were becoming increasingly dry and sterile. Such was the general feeling when Ulloa visited the coast of Ecuador in 1736. If there should be a continuation of rainy seasons such as 1939, 1941, and of more recent date, the trend would be toward a much less arid climate, but in the long run, such protracted dry and rainy periods probably tend to even up.

The garuas from late May to December are of substantial regularity, and upon these fine mists, in addition to the winter rains, is based the agriculture (maize, beans and cucurbits) of the moist parts of the coastal area from Colonche to Cape Pasado, and the production of tropical fruits, ivory nuts and toquilla straw for Panama hats. The sporadic heavy rains along the coast southward are a luxury for grazing; but they have little permanent effect on the stabilized sparse population of the dry areas. The garuas, on the other hand, determine the economic status of the inhabitants of the moist areas. With occasional partial failure of the garuas—such as occurred in the latter part of 1941—the population is reduced to abject poverty.

Eggers, who lived for several years at El Recreo, has given the best account of the region (1894, p. 270–273). From July to September, 1893, there were 245 hours of sunshine, an average of 2½ hours a day. This region of the garua affords vegetation an unbroken period of moisture in addition to the rainy period and produces conditions greatly different from those of the dry zone. [Such moist areas are shown on Eggers' map as (1) occupying the stretch of shore from Punta Ayangue to Salango, (2) part of the embayment south of Cape San Lorenzo and opposite La Plata Island, and (3) the region just south of Cape Pasado including Bahia de Caraquez and El Recreo]. Garuas are most frequent at night and in the morning. About noon the air becomes dry, but the sky remains overcast, whereas in the rainy season the sky is bright between rains. The mists are brought in by prevailing southwesterly winds and maintained at a definite height above the southwest-facing desert until checked and precipitated in the mountains.

Average temperature of the southern part of the coast is 23°–24°C. (according to Wolf, p. 389), but increases inland. On Puná Island, influenced by warm waters of the Guayas River, it is 25°. In Guayaquil it is 27°, with a maximum of 35° (95°F.). At Salinas, (Mar. 25–April 18, 1941) the temperature was normally 80°F. in the early morning, becoming 83°F. in the early afternoon, on two occasions reaching 85°F. After a violent thunder storm on the night of the 13th, it remained at 69°–70°F. for the succeeding day—the only cool temperature which I experienced. Spruce, during his visit to Chanduy in the spring of 1862, had the temperature "once down to 66½°."

In 1932 Sheppard (1934, p. 17) found abnormal migrations of sea birds, observed also in 1925–26, which penetrated inland as far as Huigra on the lower slopes of the Andes. The heaviest rains came from the sea with the wind south and southwest, the violent electrical storm of March 26 bringing 5.2 inches of rain. In January, before the rains began, the heat was unbearable (cf. fig. 5) and there was an unusual amount of sun. First rains came from the direction of the Cordillera of Colonche on the fifth of January. In February (p. 23) there was a relatively dry period (*veranillo*) in Ancon. From March 10–24 it was hot again, and the ocean temperature at La Libertad reached 85.5°F. By the middle of April, the temperature of both air and water had subsided. Total rainfall (Jan.–April, 1926) was 30.07 inches; in Guayaquil, for the same period, it was 67.91 inches.

During the rainy season of 1941 at Salinas, winds always shifted to the normal southeast-southwest sector in clearing weather. Most of the rains came

stayed to find where the equator intersected the coast; the others left for Tumbez on the 13th. Guayaquil was first located in 1535 on the Bay of Charapoto, a little north of Montecristi, then moved to the west bank of the Guayas River. Ulloa notes that the savannas in the neighborhood of the new city were not to be traveled over either on foot or horseback during the winter, for, being a spongy chalk, the level ground everywhere became one vast slough; also that the heat equalled that of Panama or Carthagena, and that during this season the elements, insects, and vermin seem to have joined in a league to incommode the human species.

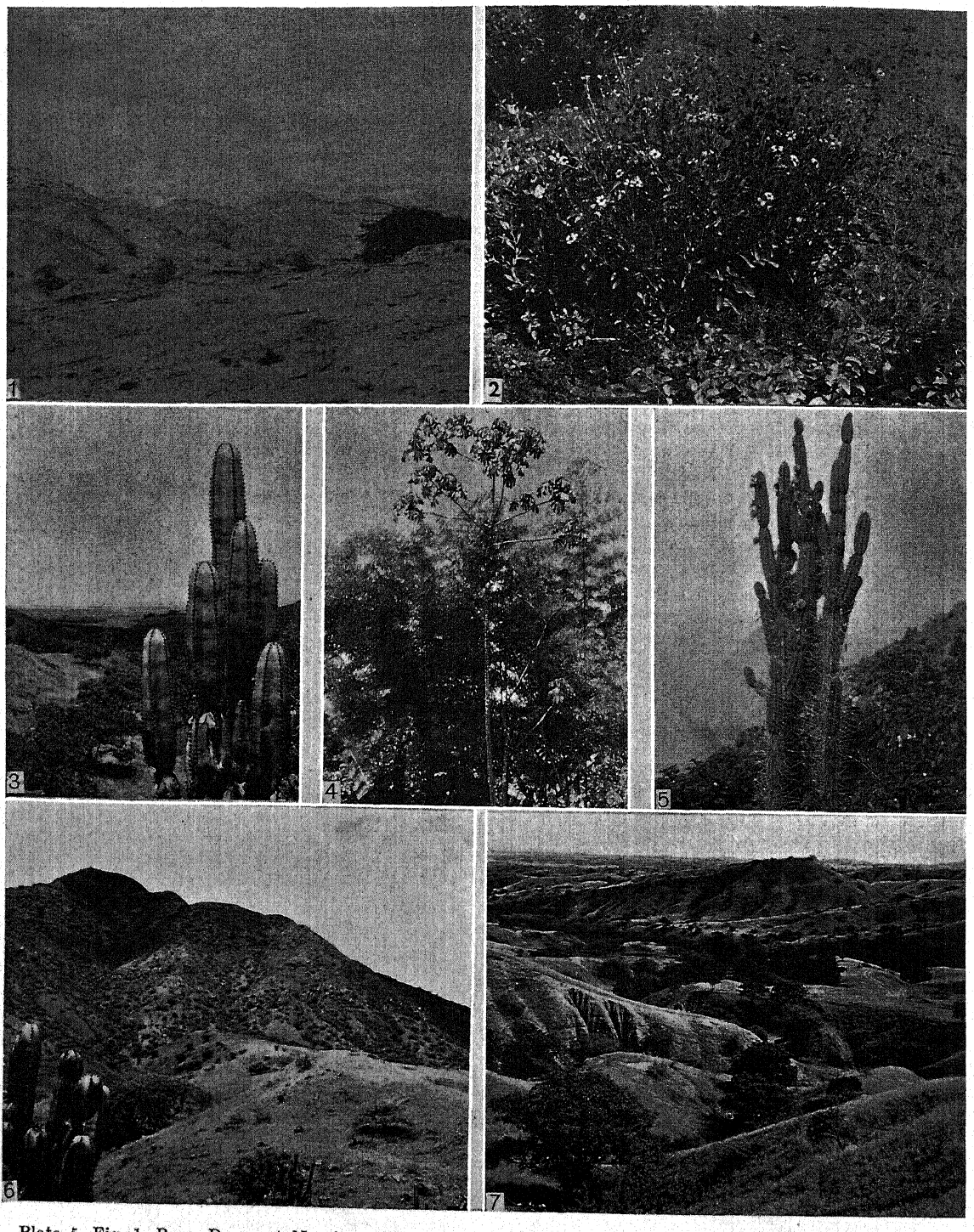


Plate 5. Fig. 1. Peru. Dunes at Negritos.—Fig. 2. Peru. Negritos, *Encelia canescens*.—Fig. 3. Peru. La Brea, *Cereus macrostibas*.—Fig. 4. Ecuador. Manglaralto, Cecripia.—Fig. 5. Ecuador, Salinas, *Cereus Cartwrightianus*.—Fig. 6. Peru. Cerro Prieto.—Fig. 7. Peru. Bad lands, e. of La Brea, *Loxopterygium* trees.

from the north, but some moved in from the southwest. At no time was there any wind from the east.

NORTHERN PERU.—The climate of northwestern Peru is similar to that of western Ecuador, except that winter rains are too irregular to produce a forest, and the amount of garua is small except upon the very summits of the Amotape Mountains. The result is that, while the mountains near the Ecuadorean coast are covered with an impenetrable tangle of vegetation [Pl. 4, fig. 3], the Amotapes are al-

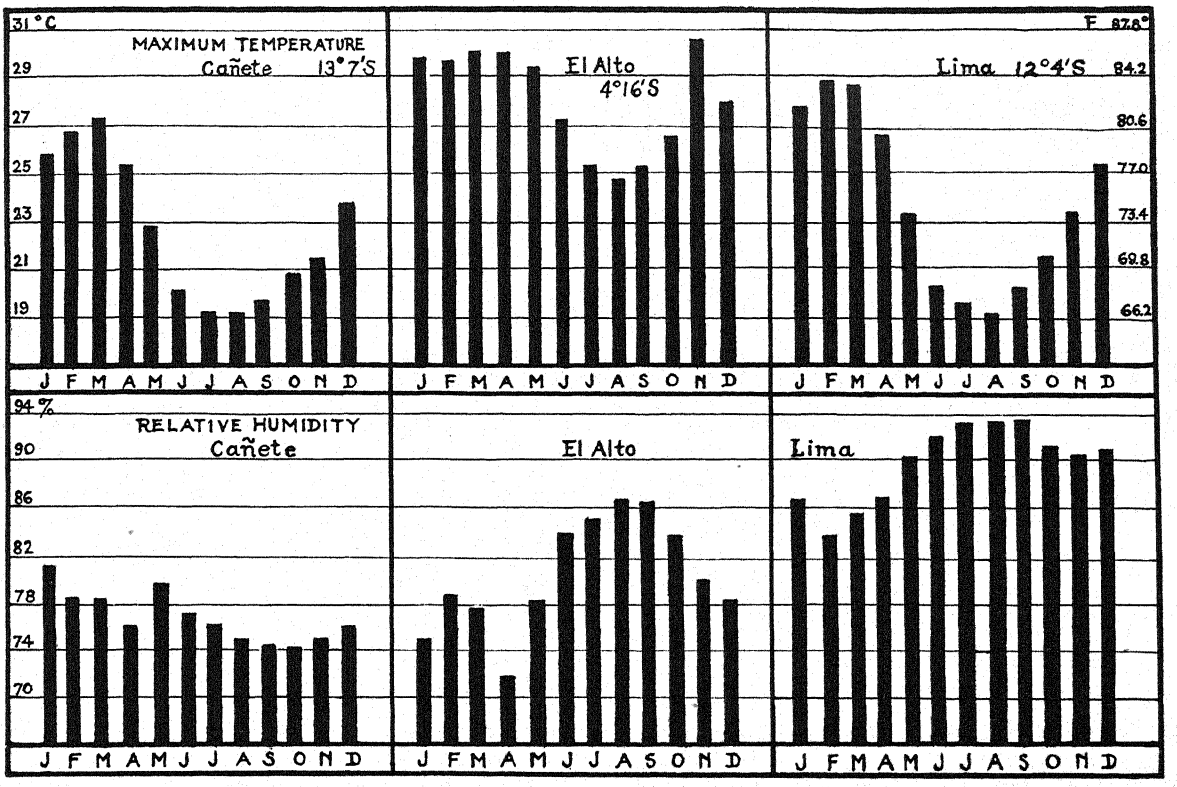


Fig. 8. Average maximum monthly temperatures and relative humidity at three stations along the Peruvian coast: El Alto (4° 16′ S, alt. 295 m. in Amotape Mts.); Lima (12° 5′ S, alt. 251 m.); Canete (13° 7′ S, alt. 36 m.). Data from Vogt (p. 11): "Stations chosen represent different elevations and different distances from coast." The graphs show variable local climates due to proximity to the ocean and to mists, as well as to latitude.

most bare, even at 3000 ft. [Pl. 5, fig. 6]. Spruce, writing on March 27, 1863, found Piura (p. 325) one of the dryest places in the world, and one of the hottest, with temperatures ranging from 83°–89°F., from December to April.

Bruns (p. 8) gives for Lima (12° 5′ S) a yearly average of 19°C. (fig. 9). The warmest month is February with an average of 23°; the coldest months are July and August with 15.9°.[9] East winds bring no rain to the coast, because it is precipitated on the terraces of the eastern Andean slopes; thus, Arequipa (elevation 2300 m.) has rainfall with east winds. The coast would remain almost wholly without vegetation, if strong garuas did not appear in the months from April to October. This transient vegetation is known as the "Loma Flora" from its development in southern Peru (cf. Weberbauer, 1911, pp. 134–149, and Johnston, 1929, pp. 6–7). Traces of it are found on the summits of the Amotape Mountains in August—its farthest northward extension. According to Bruns, the dampness of these clouds suffices to change the dust of summer into moist dirt. It is not sufficient to wash the dust from leaves of plants. The garuas form over the ocean and move inland with the wind, the moisture being precipitated unevenly depending upon relief and distance from the ocean. Thus there lies between the periodically green hills and the mountains a stretch of desert almost without vegetation, which obtains no atmospheric moisture from either western or eastern air currents. This desert covers the hilly landscape up to the foot of the Andes and follows up on to the lower slopes of the mountains. Its breadth varies from 56 km. in the south (Cachendo) to 150 km. in the north (Desert of Sechura). Ball (1885, p. 3) notes that the climate of the mountain slopes above Lima is not so dry as in southern Peru and northern Chile, where the high range of the Andes is more distant.

At intervals of 3–4 years in the Lomas, and of 5–12 years in the desert, there is actual rain for a short period. Weberbauer and Hettner believe these rains to be of Pacific origin; Köppen thinks they are "von Gebirge her versprengte Sommerregen." They are apparently the long-cycle rains, such as occurred in 1925, 1939, and 1941 at Salinas. Such disturbances reach far to the south and are strongly manifested even in Bolivia (Randolph, 1943). At Salinas, in Ecuador, the rains of 1925 and 1941 were only the fringe of a heavier precipitation over Guayaquil and the lower Andes. Chubb (1924, p. 20) mentions that "A failure of the Peruvian current may be expected whenever solar radiation is unusually low, as during a particularly low sunspot minimum."

[9] Vogt's (1942) graph I, for three localities on the Peruvian coast, is reproduced (fig. 8) in greatly modified form.

During 1940 and 1941 Vogt made observations on the winds on the island of Chincha Norte (near Pisco, Peru) and found a large percentage from the southwest quadrant. The year 1938 also had a large percentage of southwest winds, but not 1939. Vogt (1942, p. 17) discusses the origin of the Humboldt Current in some detail. He believes the Humboldt Current is a welling up of coastal waters, due to displacement of surface water by the southeast

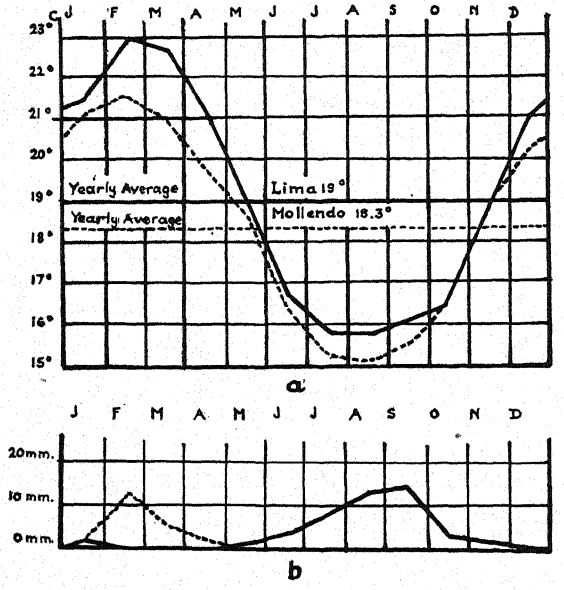


Fig. 9. (a) Temperature averages, Lima (12° 4′ S) and Mollendo (17° 5′ S), over a 6-year period, showing the slight decrease of temperature southward along the Peruvian coast. (b) Average monthly precipitation for Lima and Mollendo. August to October is the season of *garuas* or heavy fogs. The broken line for Mollendo in February and March represents fogs due to diurnal movements of air masses from mountain valleys. In both Lima and Mollendo true rains occur only at intervals of years. From Bruns (1929), figures 5, 6.

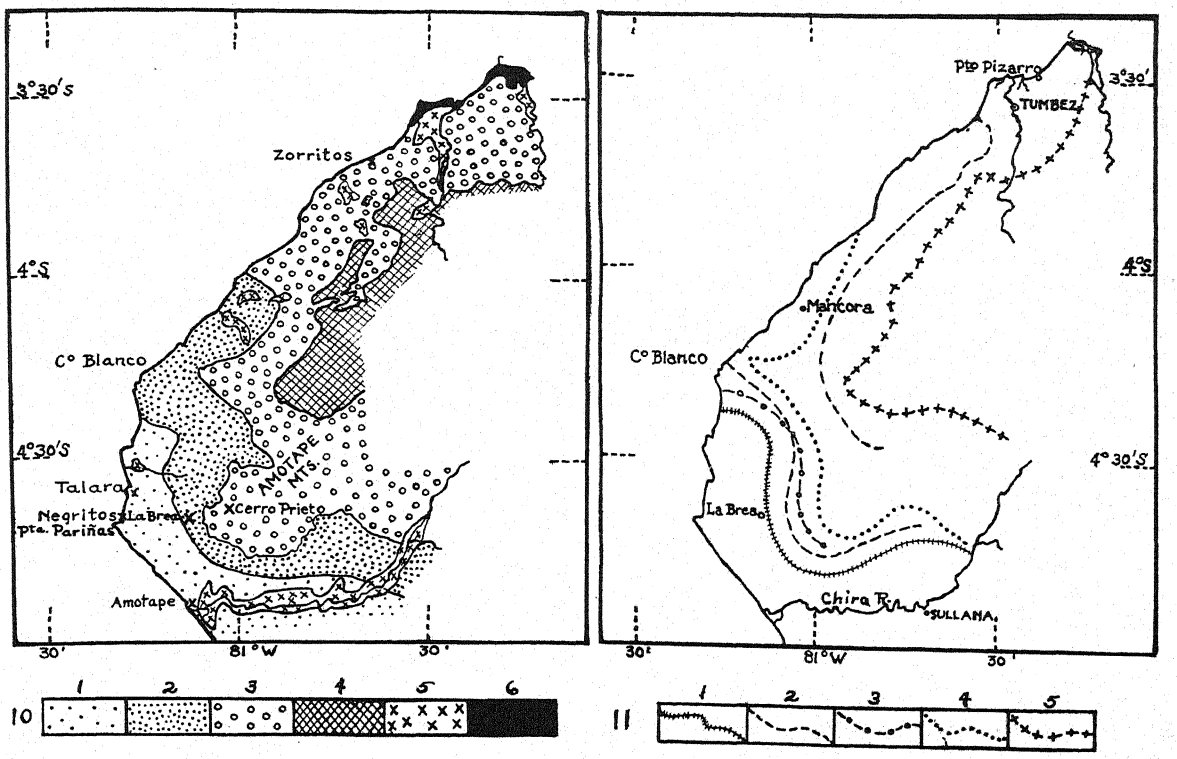
trade winds. Since the waters flow toward the open ocean and are replaced by waters from the depths, it is questionable whether the Humboldt Current really exists in the same sense as does the Gulf Stream. Perhaps it might be more exactly compared to a conveying belt which moves around a cylinder with its axis turned from the coast and which proceeds from the neighborhood of Mocha Island toward Cape Blanco. This cylinder in a sense moves counter-clockwise, bringing up waters from the depths to the surface and carrying them farther out. At the same time the entire mass is carried northward. The cleanness of the Peruvian beaches is in contrast with the beaches of eastern North America. In view of the millions of birds that annually die in this region, either on the shores, or on the open sea, one would expect large accumulations of feathers or bones. That is not the case. The birds that die in the sea are carried out to sea; those that die below the high tide level are also carried out to the open sea (p. 22). From October to February a lessened proportion of winds came from the east and adjacent quadrants, with corresponding increase in western winds and in temperature. Toward the middle of March, temperatures began to decrease and from March to June there was a constant increase of eastern winds, blowing the surface waters far from the coast and increasing the upwelling. In view of these conditions, Vogt cannot accept "El Niño"—a current from the north—as a phenomenon explaining the warm waters of 1940–1941, and believes that increased temperature of coastal waters is due to incursions of the open sea, which normally has temperatures much higher than the waters adjacent to the coast.

In February, 1931, Sheppard (1931, p. 490) noted that the waters from Santa Elena to Esmeraldes, Ecuador, became yellow and fetid, "full of plankton material, the greater part consisting of spores of some marine alga." Reference should also be made to Wolf's temperature measurements (p. 388) during his voyage to the Galapagos Islands, by which he decided that a branch of the current swung north to the Ecuadorean coast, whereas the main current continued in a westerly direction toward the Galapagos Islands. This is shown graphically by Guppy (1906, fig. opp. p. 484), and it was to this branch that Guppy (p. 494) assigned the aridity of the coast of Ecuador. If such a branch were constant, it would theoretically prevent the movement of seeds from the Ecuadorean coast to the Galapagos Islands. The main branch from Peru, on the other hand, would bear seeds directly to the Galapagos Islands. The Peru Current has been discussed in "The Oceans" by Sverdrup, Johnson, and Fleming (1942, pp. 701–706), especially in regard to changes wrought in "El Niño" years.

ZONES OF VEGETATION

Galapagos Islands.—Detailed accounts were provided for the separate islands by Stewart (1911 and 1915), an altitudinal chart of the vegetation of Indefatigable Island by the writer (1935, tables 1, 2), and by Williams (1911, pl. 21). The xerophytic transition forest is succeeded by a cloud-condensation forest composed of shrubs (*Melastomaceae*), stunted trees and many ferns. I cannot agree with my good friend, Howell (1941, p. 250), that there is a rain-forest on Indefatigable Island. On a rainy day, with water dripping everywhere, I also got that impression (1930a, p. 277), but succeeding hot and dry days, with accompanying desiccation of ferns and orchids, showed that this *Scalesia* forest is actually xerophytic.

The Galapagos Islands lie in a zone of 10–20 inches of rainfall annually (fig. 4), extending seaward from the continent. This zone extends from just below the equator in Ecuador to Tumbez and the Amotape Mountains of northern Peru, and represents the zone of "Equatorial Arid Fauna" of Chapman (1926, p. 39–40, fig. 4). For a long distance along the coast this zone is cut off by the great Peruvian Desert, but many of the Galapagos plants

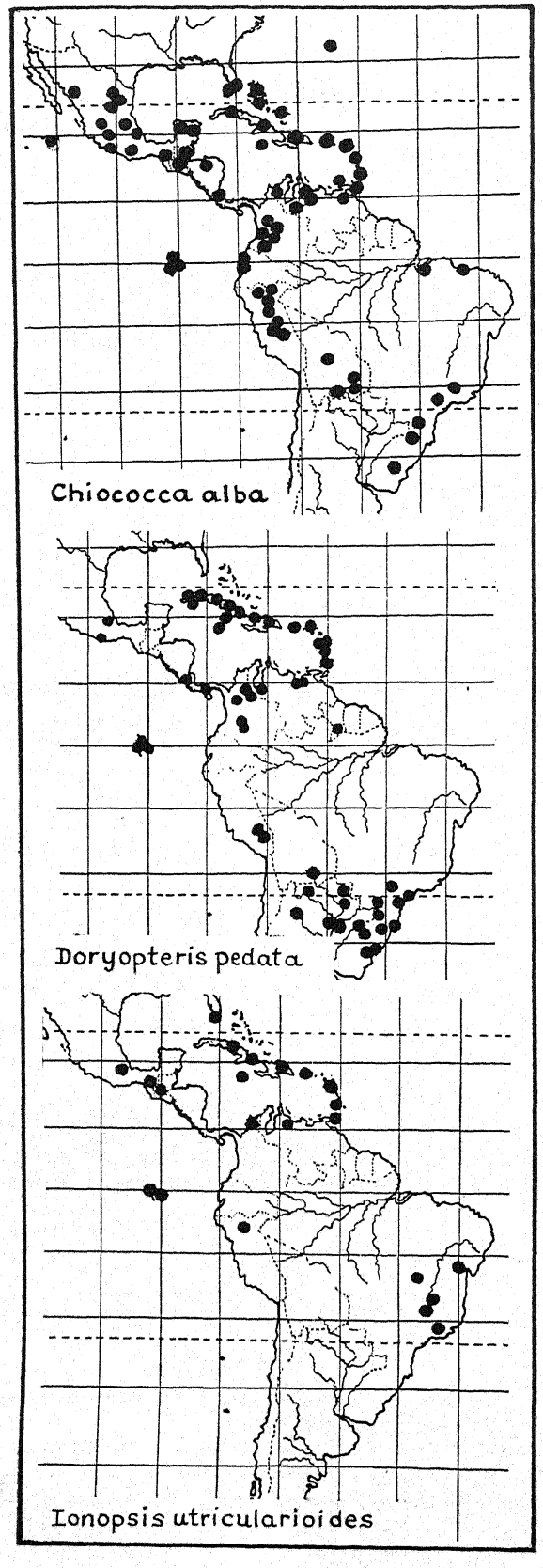


Figs. 10–11.—Fig. 10. Vegetation zones of northwestern Peru.—Fig. 11. Limits of trees on the northwest coast of Peru. Explanations in text.

(such as *Maytenus octogona*) follow the lower western slopes of the Andes far down to the region of Ica and Arequipa in southern Peru. The Tumbez region connects, through a portal of the Andes in northern Peru, with the low-lying arid plateaus of central Peru and Bolivia, and thence to the saline deserts of northwestern Argentina (cf. the distribution maps of *Jacquinia pubescens, Vallesia glabra,* and *Passiflora suberosa*), which are well illustrated by Franguelli (1941). Chapman (1926, p. 71), speaking of the connection between birds of the Arid Equatorial region and the Marañon Valley of Peru, says: "We have seen that the Porculla Pass in the Western Andes of Peru, south of Huancabamba has an altitude of only 7,078 feet. This is the lowest pass in the Andean system between southern Chile and Colombia, and it is therefore quite probable that these species [of birds] may range between the coast and valley without a break" (cf. his map, fig. 11). In summarizing the birds of the Equatorial Arid zone, Chapman (p. 73, 74) concludes that few forms of birds have been derived from the south, and that the bird distribution indicates "the former existence of a connection between semi-arid Ecuador and similar regions to the north"; that the connection has disappeared through increased rainfall following the greater elevation of the Western Andes, and that the fauna of the Equatorial Arid zone is older than that of the Marañon.

Until the dry northern coast of Colombia is reached—with its connections into Venezuela and the West Indies—plants of the Gulf of Guayaquil and the Galapagos are generally absent. An exception is seen in a few species such as *Chiococca alba* (fig. 12), *Doryopteris pedata* (fig. 12), and *Urera caracasana,* which occur in the moist parts of the Galapagos and are widespread on the continent, and which in Peru are confined to the damp trans-Andean region. The relatively few mountain species of the Galapagos seem to be associated with the little-known mountains of western Ecuador; with the Andes of Colombia and Ecuador they seem to have nothing in common.

No botanist has ever visited the Galapagos for a second time, and the bewildering variability of species has received almost no attention from an ecological point of view. One would expect unusual endemism in a group of islands occupying three degrees of latitude under peculiar climatic conditions. I am not convinced, however, that the actual percentage of endemic species is greater than occurs in the arid strip bordering the Gulf of Guayaquil in Ecuador and Peru, with which the flora of the Galapagos Islands is so intimately bound. Many species described by Hooker in 1847 have not been reexamined. Of the species listed by Stewart (1911), I would consider the following not to be endemic. Their relationship or further distribution is mentioned in the following list:



Bouteloua pilosa.	Ecuador.
Chloris anisopoda.	Ecuador and Peru.
Cyperus brachystachys.	=*C. pycnostachyus.* Mexico.
Cyperus rubiginosus.	=*C. elegans* (fig. 15).
Iresine Edmonstonei.	Ecuador.
Alternanthera echinocephala.	Peru.
Alternanthera flavicoma.	Peru.
Alternanthera halimifolia subsp. *macrophylla.*	Ecuador and Peru.
Alternanthera Helleri.	Peru.
Alternanthera rugulosa.	Peru.
Pisonia floribunda.	Probably in Peru (cf. Svenson, 1930b, p. 231).
Cissampelos galapagensis.	A form of *C. Pareira.*
Oxalis Cornelli.	Ecuador and Peru.
Kallstroemia adscendens.	Ecuador to Argentina.
Castela galapageia.	Questionably distinct from *C. erecta* and *C. fistulosa* (cf. Cronquist, Jour. Arn. Arb. 25:127. 1944).
Bursera malocophylla Rob.	Not distinct from *B. graveolens.*
Croton Scouleri.	=*C. rivinaefolius.* Ecuador.
Euphorbia viminia.	Bahama Islands.
Maytenus obovata.	Ecuador and Peru.
Cardiospermum galapageium.	A form of *C. Corindum.*
Abutilon depauperatum.	=*A. umbellatum.*
Waltheria reticulata.	*W. mollis.* Ecuador and Peru.
Passiflora lineariloba.	=*P. suberosa.*
Psidium galapageium.	Revillagigedo Islands.
Vallesia pubescens.	=*V. glabra.*
Evolvulus simplex.	Ecuador and Peru.
Ipomoea galapagensis.	=*I. triloba* (cf. Hallier f. Jahrb. Hamburg Wiss. 16: 45. 1898).
Ipomoea Kinbergi.	=*I. incarnata.* Colombia, Ecuador, Peru.
Ipomoea linearifolia.	=*I. incarnata.*
Tournefortia strigosa.	=*T. psilostachya.* Ecuador.
Cacabus Miersii.	=*C. prostratus.* Ecuador and Peru.
Elaterium cordatum.	Ecuador.
Acanthospermum microcarpum.	Ecuador and Peru.

To me, *Malvastrum dimorphum* Howell, Proc. Calif. Acad. ser. 4, 21: 331. 1935, appears to be the same species as *Sida depressa* of the Ecuadorean coast. *Drymaria monticola* Howell (op. cit., p. 329) was described from the summit of Mt. Crocker, Indefatigable Island, and differs from *D. cordata* in having smooth pedicels. Material which I collected (no. 134) on the slopes of the mountain has glandular-papillose pedicels and does not deviate from continental specimens of *D. cordata.*

Though the flora of the Galapagos Islands is closely bound up with that of the Ecuadorean-Peruvian coast, some connections occur with the vegetation of the Mexican coast and with the Caribbean region. Generalized terms such as "West Indies," "Mexico," and "South America," are frequently used in discussing the distribution of Galapagos plants, but they are too vague. In order to get down to facts, I

Fig. 12. Distribution of *Chiococca alba, Doryopteris pedata, Ionopsis utricularioides.*

have prepared a series of maps (fig. 12 to 20) showing typical geographic ranges of species of the Galapagos Islands and the mainland, with lists of species occupying these various ranges. In this respect it is necessary first to review the relationship of the Galapagos Islands with some other Pacific islands.

Of all the isolated Pacific islands the Revillagigedos are closest to the Galapagos in their flora. The two groups of islands have about 21 species in common, practically all wide-ranging weedy tropical plants. The only noteworthy species are *Psidium galapageium* and *Scaevola Plumieri,* the former having perhaps been derived from a common extinct mainland stock. *Scaevola Plumieri,* a West Indian plant, is known on the Pacific shores only from the tip of the peninsula of Lower California, on Clarion Island, the Galapagos, and the coast of Ecuador (fig. 14). To these two should be added *Cyperus pycnostachyus* (cf. fig. 16, and explanatory note). Johnston (1931, p. 35) states that the shrubby *Erigeron* species of the Revillagigedos suggest those of the Galapagos, but he suspects that the similarity is due to similar climate responses of related mainland stocks.

The problem of insular floras, well stated by Johnston (1931) in his account of the Revillagigedo Islands, involves two theories: first, that winds, ocean currents, and living organisms are completely effective in introducing plants, and that the flora is of recent origin; second, that former land connections may be involved, and that the flora goes back to past geologic times. The Revillagigedos lie under the influence of the North Equatorial Drift and California Current, which perhaps bring in plants from the coast of lower California. The weak summer current from Central America and southern Mexico would seem to have little influence on transportation of plants. The characteristic endemics of Socorro—the easternmost island—occupy sheltered moist ravines away from the sea. The relatives of this flora are not on the adjacent mainland but in southern Mexico, Central America, and the West Indies, in genera such as *Polystichum, Adiantopsis, Asplenium, Sporobolus, Paspalum*, Cattleya, Pleurothallis*, Peperomia*, Rubus, Rhamnus, Ilex*, Meliosma*, Oreopanax, Forestiera, Vernonia*,* and *Eupatorium*.* [Genera with endemic species in the Revillagigedo Islands are marked by an asterisk.] The meagerness of the upland flora in the Galapagos Islands is in striking contrast. Johnston concludes that in a former period, under moister conditions, the Revillagigedos were part of a larger land mass extending east and west and connecting with the Mexican mainland in Jalisco or Colima, and that diastrophism has separated the islands from the mainland, deepening the ocean bed and forming the Gulf of California.

Hemsley (Biol. Centr.-Am. 4: 233. 1887) published a short list of Mexican and Central-American species that occur also in the Galapagos Islands, as follows: *Bursera graveolens, Acacia tortuosa, Conocarpus erecta, Cuphea balsamona, Mentzelia aspera, Turnera ulmifolia, Mollugo verticillata, Vallesia glabra, Hyptis capitata, Salvia occidentalis, S. tiliaefolia, S. micrantha, Boerhaavia hirsuta, Phyllanthus carolinensis.* "This short list," he says, "is exceedingly interesting as showing a westward extension of the American flora. Some of the plants named may be of recent introduction through human agency; but the bulk of them may be regarded as indigenous in the sense of having reached the islands independently of man. Such a direct relationship to the American flora does not exist in any of the other Pacific Islands." With the exception of *Bursera graveolens* and *Vallesia glabra,* this list seems to me to contain only widespread weedy plants that have little geographical interest.

Cocos Island lies some four hundred miles northeast of the Galapagos Islands. It is completely covered by rain forest, and except for about eight species of widespread ferns (Svenson, 1938, p. 305), the flora has practically nothing in common with that of the Galapagos Islands.

Only two collections of Galapagos plants (*Portulaca lutea* from Daphne Island and *Ipomoea campanulata* from Iguana Cove on Albemarle), represent species of the mid-Pacific region, unknown on the American mainland. Gulick (1932, p. 415) mentions a snail, *Tornatellides chathamensis,* which has evidently been derived from the Hawaiian-Polynesian zone.

Hooker (1847) considered the new species of plants as allied to those of cooler parts of America, or of tropical uplands. In summing up the possibilities of origin (p. 250–256), he divided the Galapagos flora into two types: the West Indian (including Panama); and the Mexican or temperate American type. "The Mexican type (from the affinities of the remarkable Compositae) includes those whose nearest allies belong to Mexico or the higher levels in Colombia, or to the lower latitudes of the Southern United States, California or Chile." Hooker had, nevertheless, a good idea of the influence of the Humboldt Current upon the flora of the Galapagos Islands. Thus (p. 255): "Had this body of water flowed along more fertile coasts than the desert shores of Peru, the flora of the Galapagos might have presented a very different appearance. On the other hand, let it be borne in mind that the sterility of the coasts of this archipelago is peculiarly unfitted to the propagation of any but the most vigorous weeds of the tropics; had it been otherwise, it cannot be doubted that the palms, canes, and bamboos (which are probably originally from the Guayaquil River, and taken up by the north edge only of this current), would have vegetated here as well as in Cocos Island, only 300 miles to the north, whose vegetation is of tropical luxuriance." The similar difficulties in "rafting" of animals have been well discussed by Barbour (1916).

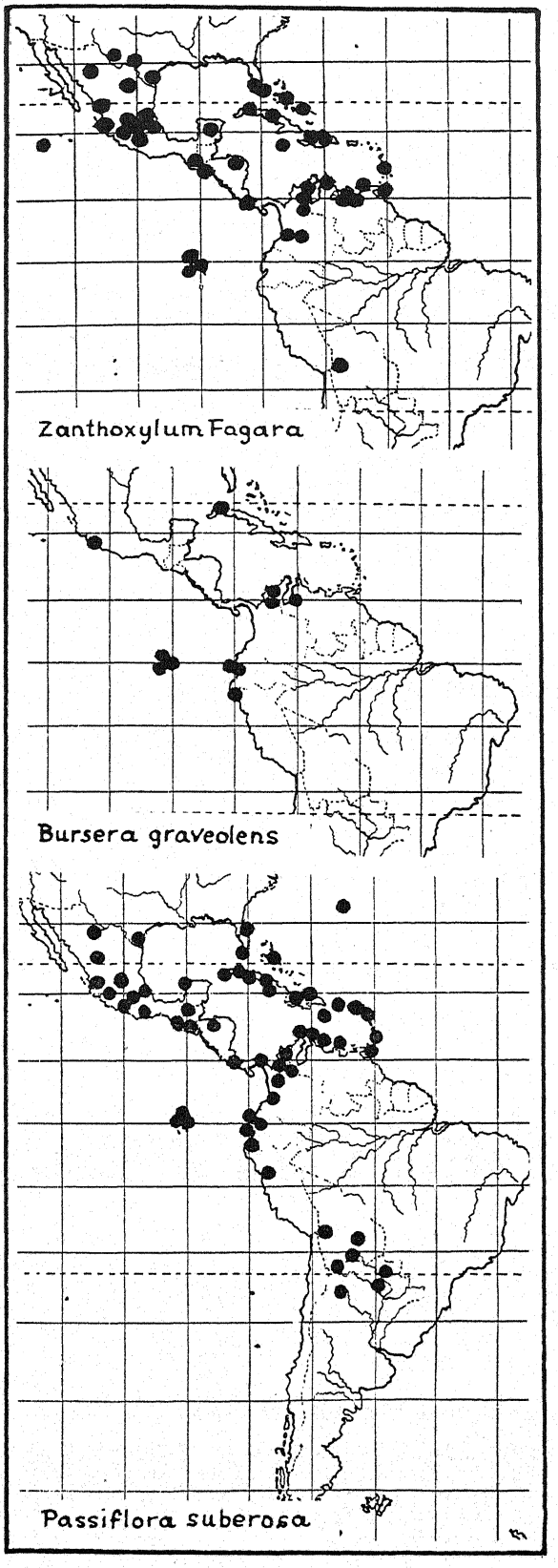
Robinson (1902, p. 239) saw that nearly all the plants of the Galapagos Archipelago were identical with, or related to, species of the Pacific slope from Lower California to northern Chile, the xerophytes resembling those of the deserts of southern Peru

and drier parts of the Andes, and the mesophytes corresponding to plants from Ecuador to southern Mexico. With the West Indies he could find no close relationship, though some affinity with the flora of the "lower slopes of Colombia" [i.e., the arid northern coastal region]. "Many cases could now be cited to show that the endemic plants of the Galapagos Islands, far from forming a marked or peculiar class, are often the nearest allies of species or varieties which are common to the islands and the continent. . . . Moreover, the endemic forms show all grades of differentiation from their continental allies; some are well marked specific types, or others mere varieties, while still others are scarcely distinguishable forms."

Theoretical aspects of the problem of island-to-island variation have already been discussed. One practical difficulty in Galapagos exploration is the great hazard in landing, except at a few sheltered spots. These landing places bear no relation to the moist or arid regions of individual islands. As a consequence, reported vegetation for any given island has often represented only that of the landing site. Lack of water and the difficult terrain have not encouraged wandering. This situation has certainly been true for the early collections of plants; it may also have been true for bird collections. Exception should be made for the moist areas of the larger islands, where trees provide shade from the sun, and where tortoise hunters or colonists had sometimes cut a trail to the more elevated regions. The botany of these areas is even now known only from a thin swathe extending from the landing place—as at Villamil on Albemarle Island, or Academy Bay on Indefatigable Island, or Wreck Bay on Chatham Island—upward to the interior. If the vegetation of any of the large islands could be known from a series of radial transects, the species problem of the Galapagos would be much better understood, at least from the point of view of "dwarfing."

By "dwarfing" I mean chiefly the reduction in leaf surface, which is the most characteristic change that continental species undergo in the Galapagos Islands. This reduction is, in general, of a progressive nature in conformity with ecological zones. For example, *Croton Scouleri* [*C. rivinaefolius* of the Ecuadorean coast] occurs on the mainland only in the large-leaved forms (var. *grandifolius* and var. *brevifolius*). At Academy Bay on Indefatigable Island, I observed a progression from the large-leaved phase (in the moist forest at 400–600 ft.) through typical *C. Scouleri* (to 250 ft.) to the narrow-leaved var. *Macraei* (sea-level to 150 ft.) (cf. Amer. Jour. Bot. 22: pl. 4, fig. 1a; pl. 9, fig. 1. 1935). Var. *Macraei* is known from Cowley Bay and Tagus Cove on Albemarle Island, on the lower parts of Charles Island, in the vicinity of the shore at Academy Bay on Indefatigable Island, and on Orchilla Bay on James Island. A similar series characterizes *Waltheria ovata* on the coast and in the Galapagos Islands, though the ecological situation is less well understood. The most reduced form of *W. ovata* in the Galapagos Islands is typical *W. reticulata* Hook., known from Abingdon Island; Cowley Bay, Elizabeth Bay, Tagus Cove and Villamil on Albemarle Island; Charles Island; Duncan Island at 1275 ft.; James Island; Jervis Island; and from a salt lagoon near the coast of Indefatigable Island (coll. *Riley*). Other continental species which show characteristic dwarfing are *Abutilon umbellatum, Sida spinosa, Ipomoea incarnata, Cyperus pycnostachyus, Alternanthera ficoidea, Acacia macracantha, Erythrina velutina, Tephrosia cinerea, Galactea volubilis, Crotalaria pumila, Zanthoxylon Fagara, Vallesia glabra, Elaterium carthagenense,* and *Doryopteris pedata.* The endemic *Lantana pedunculata* also shows a well-marked variation within the islands. What is the cause of this dwarfing, so characteristic of species of the Galapagos Islands? Nothing is definitely known. One possibility is that the relative absence in the Galapagos Islands of the steady drying winds which so sharply delimit the vegetative zones on the Ecuadorean-Peruvian coast (cf. discussion under *Croton rivinaefolius,* Cat. no. 171) may allow the bare existence—and there is not much competition—of these dwarfed forms on inhospitable lava flows and other arid situations. Galapagos species are in general not those of the Peruvian Desert, but of a less arid inner zone (cf. fig. 4) which touches the coast at a few points. Secondly, there is a marked stunting of trees and shrubs toward the mountain summits, which may be due to a different set of factors, such as fog and reduced sunlight. Until the Galapagos Islands are investigated from an ecological point of view, it would seem that the question of variability of plants from island to island, upon which so much statistical data has been published, would seem rather futile.

Whether the same influences that produce the variability of plant species in the Galapagos Islands also affect the distribution and speciation of birds, is unknown. Williams (1911, p. 297) mentions "the dwarfing effects of the arid regions" on the insects. Nomenclature of the birds seems to be a difficult problem, and as Swarth (1931, p. 17) in the most recent review of the subject, says ". . . . whether a given form is a species or a subspecies or whether or not it is a 'good' subspecies, is rather beside the mark, they so resolutely refuse to conform to the standards applied to continental species. . . . Whether the bewildering conditions existent among these island birds arise from the presence of factors that are ordinarily absent from the surroundings of mainland forms, or whether they are due in part to an instability in rapidly succeeding generations such as is not commonly seen elsewhere, can not be said, but I incline to the latter view." "Rigid adherence to accepted criteria for subspecific association of forms could be followed to absurd lengths." And p. 16: "Variation in all degrees of accentuation occurs among series from different islands [sometimes imperceptibly] and it is not at all easy to decide just what degree of an observed trend deserves the artificial (and perhaps unduly exaggerated) emphasis



of a separate name. Perhaps the only consistent handling of the situation would lie in giving a separate designation to the representative of each separate form upon every island, whether obviously different or not. . . . Intergradation between different extremes occurs to a bewildering degree, so as to render any system of nomenclature more or less of an artificial procedure."

For relationship between vegetation of the Galapagos Islands and the adjacent coast, and an insight into zonal distribution of the endemics, Stewart's list of plants (1911, p. 208) characteristic of the transition zone is appended, excluding the ferns. Some distributional notes, and references to the catalog in the second part of this paper, have been added.

Bursera graveolens (also in dry zone). Fig. 13.
Castela galapageia (also in dry zone). Questionably endemic.
Chiococca alba (also in moist zone). Fig. 12.
Cissampelos Pareira (also in moist zone). Widespread.
Clerodendron molle (also in dry zone). Distinct from *C. aculeatum?* Cat. no. 256.
Cordia galapagensis (also in dry zone). Endemic.
Cordia Hookeriana [*C. revoluta*] (also in dry zone). Endemic.
Cordia lutea (also in dry zone). Western Ecuador and Peru, Cat. no. 245.
Croton Scouleri var. *brevifolius* [*C. rivinaefolius*, Cat. no. 171].
Erigeron tenuifolius. Endemic.
Euphorbia viminea (also in dry zone). Bahamas.
Gossypium barbadense (also in dry zone). Western South America.
Iomopsis utricularioides (also in moist zone). Fig. 12.
Lantana peduncularis (also in dry zone). Endemic.
Lipochaeta laricifolia. Endemic.
Maytenus obovata [*M. octogona*, Cat. no. 183] (also in dry zone). Ecuador and Peru.
Pisonia floribunda (also in moist zone). Peru?
Psidium galapageium (also in moist zone). Revillagigedo Island. Fig. 16.
Psychotria rufipes (also in moist zone). Endemic.
Scalesia pedunculata (also in moist zone). Endemic.
Alternanthera echinocephala (also in dry zone). Peru. Cat. no. 86.
Tillandsia insularis (also in moist zone). Endemic.
Tournefortia rufo-sericea (also in moist zone). Endemic.
Waltheria reticulata [*W. ovata*, Cat. no. 201]. Also in dry zone.
Zanthoxylon Fagara (also in moist zone). Fig. 13.

It will be seen from these lists that there is a considerable amount of overlapping in the zones, and that the endemics, on the basis of these lists, are more abundant in the dry region. Species characteristic of the dry zone (according to Stewart, p. 207) and not incorporated in the previous list, are as follows:

Acacia macracantha. West Indies and South America. Cat. no. 131.
Aristida divulsa. Endemic.
Aristida subspicata. South America, according to Robinson.
Borreria ericaefolia. Endemic.

Fig. 13. Distribution of *Zanthoxylum Fagara*, *Bursera graveolens*, *Passiflora suberosa*.

Cenchrus platyacanthus. Endemic.
Cereus galapagensis. Endemic.
Cereus nesioticus. Endemic.
Cereus sclerocarpus. Endemic.
Coldenia Darwini. Endemic.
Coldenia fusca. Endemic.
Croton Scouleri vars. *brevifolius* and *Macraei*. Endemic.
Desmanthus depressus. Widespread in tropics. Cat. no. 141.
Discaria pauciflora [*Scutia spicata*]. Ecuador & Peru. Cat. no. 185.
Erythrina velutina. Ecuador. Cat. no. 145.
Euphorbia amplexicaulis. Endemic.
Euphorbia articulata. Endemic.
Mentzelia aspera. Widespread in tropics. Cat. no. 207.
Opuntia galapageia. Endemic.
Opuntia myriacantha. Endemic.
Parkinsonia aculeata. Widespread in tropics.
Piscidia Erythrina. Widespread in tropics.
Prosopis dulcis [*P. juliflora*]. Widespread in tropics. Cat. no. 155.
Scalesia atractyloides. Endemic.
Telanthera nudicaulis. Endemic.

Species (excluding ferns) enumerated by Stewart (p. 209) as characteristic of the moist zone of the Galapagos Islands, and not enumerated in the list from the transition zone, are as follows:

Argyreia tiliaefolia. Widespread in all tropics.
Croton Scouleri var. *grandifolius* [*C. rivinaefolius*]. Ecuador.
Epidendrum spicatum. Endemic.
Erigeron linifolius. Widespread in tropics.
Scalesia cordata. Endemic.
Urera alceaefolia [*U. caracasana*].

This last bush or small tree, with stinging hairs, is known in the Galapagos Islands only from the moist slopes of Indefatigable, James, and Albemarle Islands. It is widespread in the American tropics, with a distribution much like that of *Chiococca alba* (fig. 12). According to Grisebach (Fl. British W. I. 154. 1860), *U. caracasana* extends from Mexico to Buenos Aires. In Peru (cf. Macbride, Fl. Peru, pt. 2: 263. 1937) it is known from the Amazon side of the mountains, and extends into Bolivia and northwestern Argentina, but Hauman & Irogoyen (Anal. Nat. Hist. Buenos Aires 32: 410. 1925) question its occurrence at Buenos Aires. *Stewart* 1345, from 2000 ft. altitude on James Island in the Galapagos, has leaves acute at the base and not cordate as is customary in the species. Otherwise it is identical with continental material, both as to leaf texture and pubescence, and details of the inflorescence. This collection shows a reduction in leaf surface, which is characteristic of many Galapagos plants.

The moist zone has a poor representation of endemics as compared with the mesophytic flora of the Revillagigedo Islands. Much of the vegetation of the mountain tops consists of ferns of widespread distribution (Svenson, 1938, p. 304–306) due probably to the fact that spores are so readily carried by the winds. Of the ferns in the Galapagos Islands, *Doryopteris pedata* (fig. 12) is one of the few with an interesting or significant distribution. Of more importance is *Miconia Robinsoniana* (Melastomaceae), abundant toward the summits of Indefatigable and Chatham Islands, and (as Dr. Gleason tells me) very closely related to species of the Ecuadorean mainland. Also in the moist zone, in the vicinity of the plantation, Fortuna, on Indefatigable Island, occurs a terrestrial orchid previously referred by me to *Corymborchis*. This was obtained in flower from the same locality by *Schimpff* (no. 84) and his collection was correctly identified as *Tropidia polystachya* (Sw.) Ames, Orchidaceae 2: 262. 1908. It agrees well with the illustration in Contrib. Ames Bot. Lab. no. 1: pl. 5. 1904. Previously this plant has been known from Jamaica, Cuba, and Florida. Plants of *Colocasia esculenta* grew not far from this rather weedy orchid, and there is some possibility that it may have been unintentionally brought into the islands in recent times.

The limits of individual woody species on the southern side of Indefatigable Island—where the moist zone comes down relatively far toward the sea—have been illustrated graphically by me (1935, p. 210–211). The altitudinal distribution in this locality, excepting endemic species, affords a good basis for comparing zonal distribution on the Galapagos Islands with geographic and climatic distribution of the same species on the continent and on the Caribbean Islands.

1. Dry zone (approx. 0–200 ft.). Trees: *Bursera graveolens, Hippomane Mancinella, Cordia lutea, Croton Scouleri* var. *Macraei, Hibiscus tiliaceus, Parkinsonia aculeata*. Shrubs: *Alternanthera echinocephala, Euphorbia viminia, Gossypium Darwini, Cordia galapagensis, Tournefortia psilostachya* and *T. pubescens, Lantana peduncularis, Acacia tortuosa, Solanum verbascifolium, Cryptocarpus pyriformis, Chiococca alba*, and *Scalesia affinis*.

2. Transition and moist zone (approx. 200–1200 ft.). Trees, in order of their abundance: *Zanthoxylum Fagara, Piscidia Erythrina, Pisonia floribunda, Scalesia pedunculata, Psidium galapageium, Urera alceaefolia, Hippomane Mancinella*. Shrubs: *Psychotria rufipes, Lantana peduncularis, Tournefortia rufo-sericea, Chiococca alba, Croton Scouleri* var. *grandifolius*.

3. Mountain zone of dwarfed trees and shrubs. Trees: *Zanthoxylum Fagara, Piscidia Erythrina, Miconia Robinsoniana*. Shrubs: *Psychotria rufipes, Chiococca alba, Erigeron tenuifolius, Phyllanthus carolinensis*.

Of these trees and shrubs the most widely-distributed zonally, and therefore the most adaptable, seem to be *Zanthoxylum Fagara, Chiococca alba*, and *Piscidia Erythrina*. These are all of wide distribution in the Caribbean area and continental South America (cf. fig. 12, 13), but are not tolerant of extremely dry places in the Galapagos Islands.

Northwestern Peru.—Since this area is a key also to the vegetation zones of Southwestern Ecuador, Weberbauer's charts of plant formations (1929, pl. 1, 2) have been reproduced in modified form as figures 10 and 11. The first of these (fig. 10) shows

zones from the Amotape Mountains across the narrow coastal plain to the Pacific Ocean:

1. Woody plants (trees and shrubs) much dispersed, evergreen, maintained by subterranean water. Absence of herbaceous plants and cacti.

2. Woody plants (trees and shrubs) dispersed, evergreen, maintained by subterranean water. Some deciduous woody plants. The soil covered with herbaceous plants during the rainy months. Cactaceae rare or absent.

3. Park-like rain-green xerophytic association consisting of trees, shrubs, columnar cactus and herbs; the trees and shrubs frequently forming groups. At the end of the rainy period the herbaceous plants dry up and the larger part of the trees and shrubs lose their leaves.

4. Rain-green mountain vegetation consisting of trees, shrubs and columnar cactus. At the end of the rainy period most trees and shrubs are deciduous.

5. Algarroba (*Prosopis*) thickets, including lands under cultivation.

6. Mangrove swamps.

Figure 11 shows the limits of growth, toward the coast, of certain key species of the vegetation. It is obvious that many of the data for the ranges of these plants were obtained by Weberbauer from Bosworth's account (1922) of the geology of this region: 1. Outer limit of *Loxopterygium Huasango*. 2. Outer and inner limits of *Cereus macrostibas*. 3. Outer limit of *Bursera graveolens*. 4. Outer limit of *Cereus Cartwrightianus*. 5. Outer limit of *Bombax discolor*. Further distribution of these five species is given in the following notes:

Loxopterygium Huasango (Anacardiaceae) (pl. 5, fig. 7). Also known from the arid region of Ecuador where it is the most abundant tree.

Bursera graveolens. Palo Santo (Burseraceae). Mexico and the West Indies; the Cartagena and Santa Marta regions of Colombia, southwestern Ecuador, and the Galapagos Islands (fig. 13).

Cereus macrostibas Berger, Rep. Mo. Bot. Gard. 16: 69. 1905. *Pilocereus macrostibas* Schumann, Monatssch. Kakteenk. 13: 168. 1903. *Neoraimondia macrostibas* Britton & Rose, Cactaceae 2: 182, fig. 257–260. 1920. This enormous cactus (pl. 5, fig. 3) is found in western Peru from Arequipa and Chosica northward to the area outlined here. It is not known from Ecuador.

Cereus Cartwrightianus (pl. 5, fig. 5). An abundant cactus of southwestern Ecuador, extending southward into Peru within the influence of the Amotape Mountains.

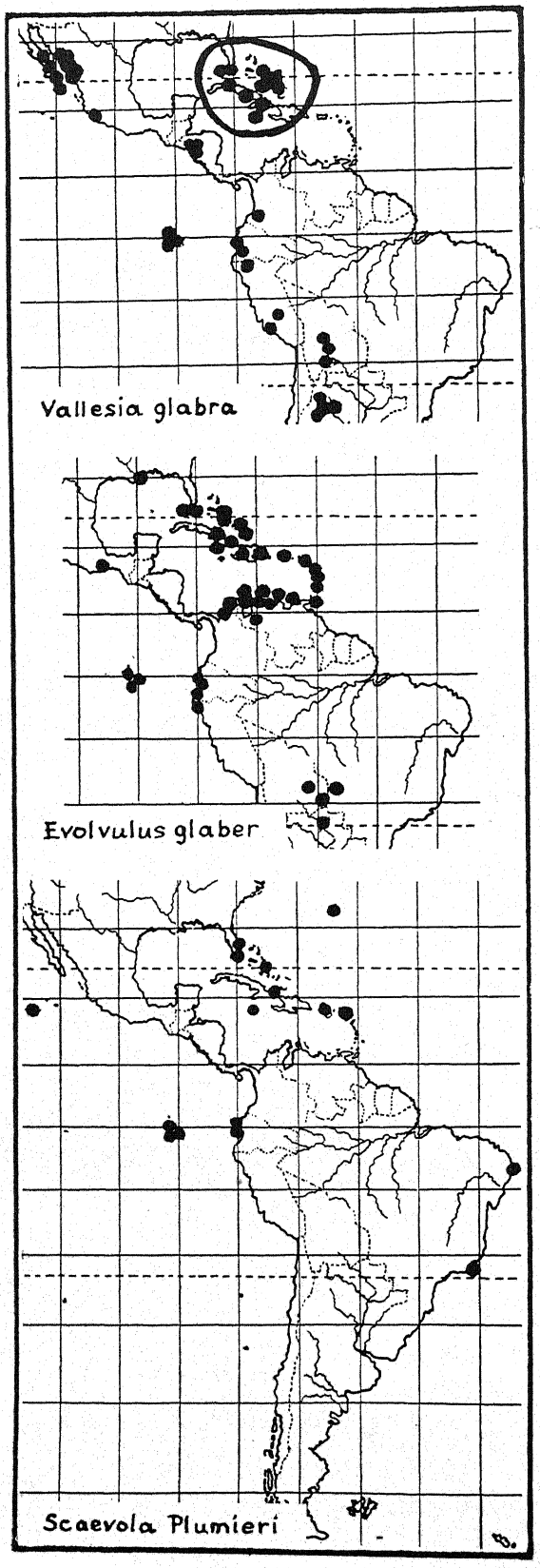
Bombax discolor (Bombacaceae). This species was described by HBK. from San Filipe, Prov. Jaen de Bracamoros (now in Dept. Cajamarca). It is characteristic of the lower Andean region, and may perhaps be the same as *B. Ruizii* K. Sch. in Engler, Bot. Jahrb. 25, Beibl. 60: 16. 1898, from Guayaquil, which would theoretically have the same geographical distribution. *B. Ruizii* is probably the large *ceiba* which extends westward from Guayaquil at the foot of the Chongon Hills.

Even in a rainy season only the dwarf yellow-flowered bushes of *Encelia canescens* (Pl. 5, fig. 2) and a few scattered algarroba trees enliven the sandy wastes from Talara to Negritos. This area (Zone 1 in Weberbauer's map) is the northward extension of the desert of Peru and Chile, and the sparse vegetation is that of the Andean foothills near Lima, or of the Peruvian littoral. Such species frequently extend southward to the coast of Chile, but seem dissociated from the herbaceous Loma Formation of southern Peru, which is dependent on the garuas that reach their height in August and September (see fig. 8 and 9b). Ball (1877) found only twelve species within more than a mile radius of Piura,[10] and there were no annuals among these, since they depend upon long roots to reach a subterranean water-supply. Bosworth (p. 314)[11] thinks that a few of the juicy creeping plants, such as are found on the seaward side of the rocks and dunes at Negritos, may depend on the light mist that is formed along the shore in the evening. Following Bosworth's account, a few miles inland from the coast the *Algarroba* appears along with the *zapote* [*Capparis* sp.], the pods of which are eaten by foxes and other desert animals; at 600–800 ft. (p. 315) is *cardo* or tree-cactus [*Cereus macrostibas*], which becomes abundant northward. After entering the mountain valleys, algarrobas appear as well-developed trees, along with the gum-tree, *bualtaco* [*Loxopterygium*]. At about 1500 ft. the giant cactus, "gigante" [*Cereus Cartwrightianus*] rises to 20–30 ft., and the *palo santo* [*Bursera graveolens*] grows to 15–20 ft. The *tupeia* [*Tillandsia* sp.], with fragrant purple flowers, is a plentiful epiphyte at about 200 ft., and the only abundant material upon which beasts can live. Moisture is more abundant on the highest summits of the Amotape Mts. and Bosworth's photograph of the top of Cerro Viento (5000 ft.) shows dwarfed leafless trees drapped with species of *Tillandsia*. According to him, the *cardo* trees reach the coast at about 25 miles north of Mancora. The *bualtaco* and *palo santo* also occur near sea-level at the north end of the desert.

Occasional algarroba bushes (*Prosopis juliflora*) are the only green plants which I saw on the eroded Tertiary rocks that come down abruptly to the sea at Negritos (Pl. 5, fig. 1). Other species are represented by widely scattered individuals in the gullies, all with gray or pallid leaves, brightened somewhat by the orange fruits of *Grabowskya* and by the minute pink flowers of *Boerhaavia verbenacea* (Cat. no. 110) and the orange flowers of *Hoffmannseggia viscosa* (Cat. no. 148). Small mats of *Alternanthera* lie prostrate in the sand; otherwise no vegetation exists until clumps of *Batis maritima* and *Trianthe-*

[10] *Tephrosia cinerea, Coldenia paronychioides, Hoffmannseggia viscosa, Galvesia limensis, Prosopis limensis, Lippia reptans, Acacia tortuosa, Alternanthera halimifolia* (*Telanthera densiflora*), *Acacia* sp., *Alternanthera peruviana, Encelia canescens, Capparis scabrida* (acc. to Weberbauer, 1911, p. 3).

[11] Bosworth gives a good general account of the vegetation.



ma Portulacastrum are reached at the water's edge.

Going inland for a few miles on the sandy tablazos, one finds in favorable localities a meager assemblage of unusual species, such as *Proboscidia peruviana* (Cat. no. 282) (known only from this sandy region of Peru), the erect silvery stems of *Alternanthera truxillensis,* and stunted bushes of *Lantana Sprucei.* Farther inland the broadened outlets of the quebradas usher in the second vegetative zone of Weberbauer, a continuation of the coastal zone of southwestern Ecuador, though the amount of vegetation is much reduced. In Quebrada Ancha, near La Brea, the algarroba becomes frequent, and small *Loxoterygium* trees appear. Most prominent are *Capparis* bushes, *C. angulata, C. cordifolia,* and *C. mollis* (Cat. no. 127) with evergreen leaves. Upon them climb *Luffa Astori* (Cat. no. 301), a species similar to *L. operculata* but with larger flowers. As on the Ecuadorean coast, fruits of the preceding year remain hanging in the trees and bushes. Mingled with the *Luffa* are slender vines of *Ipomoea aegyptia* (Cat. no. 230) with 5-parted leaves and white flowers (pl. 3, fig. 3); the pink flowers of *I. Nil;* and closer to the ground, *I. dumetorum* with white to pale blue flowers. A sparse carpet of annual grasses, such as *Aristida adscensionis, Chloris radiata,* and *C. virgata,* provides a meager food for live-stock. Here also are the weedy species of *Pectis—P. linifolia* (Cat. no. 317) and *P. diffusa* and two other weed-like composites, *Lagascea mollis* and *Simsia Dombeyana.* As one proceeds inland along the quebrada, tangles of the blue-flowered *Jacquemontia secunda* (Cat. no. 241) and the strong-scented *Acacia Huasango* (Cat. no. 130) form little thickets among the gravels, and the algarroba bushes frequently show brilliant scarlet patches of a mistletoe, *Psittacanthus. Cordia lutea* (Cat. no. 245) is one of the most abundant bushes. Bosworth says that the glue-like substance in the fruit is used in making kinky hair straight. Of herbaceous plants there are none with large flowers except a purple-flowered mallow, *Cienfuegosia heterophylla* (Cat. no. 191), a species widely distributed in the American tropics. Most of the herbs have small yellow flowers: *Portulaca Haughtii* (Cat. no. 118), *Crotalaria pumila* (Cat. no. 139), *Stylosanthes sympodialis, Oxalis Cornelli.* Some are dull blue, as *Tephrosia littoralis, Desmodium molle,* and *Evolvulus simplex.* In *Mimosa albida* the flowers vary from deep pink to nearly white. This desert flora which appears after the rains has been described as of some elegance, but it seems poor in both color and the number of species represented. Most of the herbaceous species of this zone are also seen in the drier parts of the Galapagos Islands. When the rocky walls of the quebradas are reached, the vegetation becomes more colorful, with the bright blue flowers of *Browallia elata* (Cat. no. 267) and *Evolvulus piurensis* and the white button-like heads of *Spilanthes alba* (Cat. no. 322) appearing in thickets of *Cordia polyantha* and *Piquieria*

Fig. 14. Distribution of *Vallesia glabra, Evolvulus glaber, Scaevola Plumieri.*

peruviana, and with *Jacquemontia secunda* and *Lantana Sprucei* (Cat. no. 258) climbing over the exposed rocks. At the head of the Quebrada Mogollon are large beds of quartz sand,, and on the surrounding rocks four of the characteristic plants of the Galapagos Islands: *Boerhaavia tuberosa, Scutia spicata, Capraria peruviana,* and *Cryptocarpus pyriformis,* together with the ubiquitous *Cardiospermum Corindum.*

The vegetation has a more dispersed character than in any portion of the Ecuadorean coast, except possibly in a small portion of the Santa Elena peninsula. In ascending Cerro Prieto (pl. 5, fig. 6) (3000 ft. alt.) some species are encountered on the dry rocky slopes which I did not see in Ecuador: *Cottea pappophoroides* (a grass with pale pink flowers), *Phyllanthus orbiculatus,* the melon-like *Cactus Townsendii,* and the very localized *Eremocharis dissecta.* At the summit is a patch of grass in which the shrubby *Brachistus Haughtii* and the pink-flowered composite, *Onoseris integrifolia* grow; and just below, on a ridge, is *Solanum amotapense.*

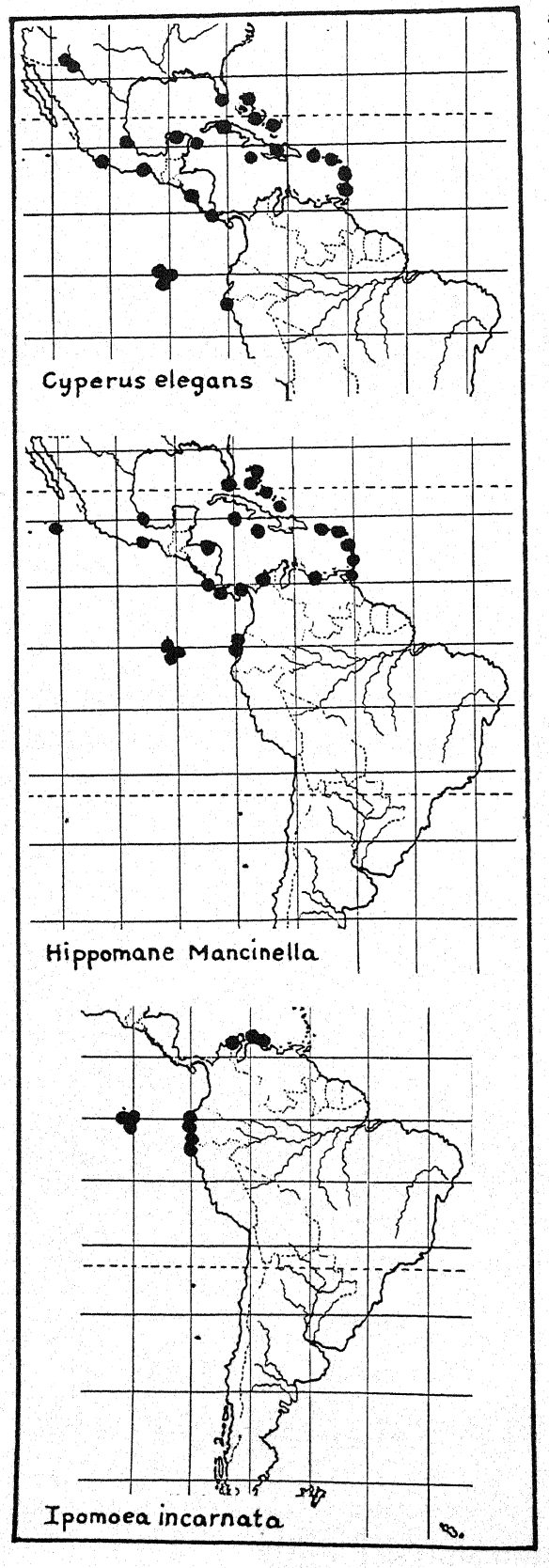
Southwestern Ecuador.—The most barren portion of the Ecuadorean coast is the peninsula of Santa Elena, together with such adjacent projections as Punta Centinela. A few scraggly algarrobas seem to be the only native trees in the low area east of the Puntilla, except for those planted around water holes. In the neighborhood of Santa Elena there are isolated dwarf trees of *Jacquinia pubescens,* and north of Ancon, *Loxopterygium* forms groves on the low hills.

Annual grasses, the same species as in the Amotape region of Peru, sparsely cover the thin sandy soil, and from the flat surface of the ground isolated rounded shrubs rise to the height of four or five feet. These shrubs are *Maytenus octogona, Scutia spicata, Cordia lutea, Capparis avicennifolia, Cryptocarpa pyriformis, Vallesia glabra,* and *Acacia Huasango.* The outer dunes which face the dry southwest winds have no vegetation except occasional mats of *Pectis arenaria* or *Sporobolus virginicus.* On protected inner dunes or margins of salt flats, *Ipomoea Pescaprae* sends out long runners, and the sands are studded with *Coldenia paronychioides, Boerhaavia erecta, Pectis diffusa,* dwarf specimens of *Cyperus aristatus, C. esculentus,* and *C. fugax, Eleocharis atropurpurea,* and *Hemicarpha micrantha.* Here and there, where the soil is a trifle richer, are *Amaranthus Haughtii* and *A. urceolatus, Portulaca Haughtii, Sida ciliaris* and *S. Weberbaueri, Cacabus prostratus,* and *Acanthospermum microcarpum.* Occasional species are *Monnina chanduyensis, Polygala alopecurus, Cassia nictitans, Aeschynomene pluriarticulata, Zornia diphylla,* and *Marsypianthes Chamaedrys.* The borders of the small ponds formed in the raniy season (pl. 3, fig. 1) are disappointing in the meager number of species to be found at their borders: *Marsilea ancylopoda, Cyperus compressus* and *C. oxylepis, Cressa truxillensis, Alternanthera halimifolia, Ammania auriculata,* and *Isocarpha microcephala.*

The dry southwest winds cause a remarkable difference between the vegetation of the southern part of the Puntilla (Pl. 1, fig. 1) and the small area at the north side under the protection of sheltering cliffs. On the south side straggling shrubs of *Cordia lutea, Waltheria ovata,* and *Heliotropium angiospermum,* are scarcely able to rise from the ground. On the north slope is a flora not to be found until one reaches the protected slopes of the Cerro Ballenita near Santa Elena, or the hilly region with ravines north of Ancon. This flora is also characteristic of the drier environments of Guayaquil. A half dozen small *ceiba* trees (probably *Bombax Ruizii*) on the north slope show the protecting influence of the cliffs, for *ceiba* trees do not appear eastward until one passes Baños San Vicente. The chief plants of this sheltered region of the Puntilla are as follows: *Caesalpinia corymbosa, Pithecolobium candidum, Abutilon umbellatum, Buettneria glabrescens, Calonyction Pavonii, Phaseolus pedunculatis, Cordia polyantha, Browallia elata, Elytraria imbricata, Cyclanthera leptostachya.*

From the crest of the rocks long streamers of *Lycium Tweedianum* hang down over the cave openings that represent the coast line before elevation. Within range of salt spray grow *Ruellia floribunda, Boerhaavia coccinea, Alternanthera pubiflora, Oxalis Cornelli, Euphorbia lasiocarpa,* and *Ipomoea incarnata.* In this gravelly soil are several plants already cited from the intermediate zone of Peru: *Mimosa albida, Ipomoea aegyptia, I. dumetorum, I. Nil,* and *Jacquinia secunda.*

An inner zone is reached east of a line from Ancon to Santa Elena, where the black loam becomes impassable in the wet season, and great savannas stretch twenty miles to the eastward. In early February, these are covered with countless white "lilies" (*Hymenocallis quitoensis*) and later by tall stands of the grasses, *Pennisetum occidentale* and *Eriochloa pacifica.* A few miles east of Santa Elena, the candelabra-like trees of *Cochleospermum vitifolium* are first seen, together with the shrubby composite, *Wedelia grandiflora,* which reaches the coast at Punta Ayangue. *Croton rivinaefolius,* perhaps the most abundant shrub on the Galapagos Islands, extends eastward to Guayaquil from the Santa Elena-Ancon region, and reaches the seacoast at Punta Ayangue and Manglaralto. It is also on the higher parts of the Chanduy Hills. In this zone cactus (*Cereus Cartwrightianus*) and adjacent trees are often wholly covered by *Luffa.* The *Hymenocallis* also occurs in sheltered spots on the Cerro Ballenita at Santa Elena, and Dr. Murphy has photographed it on La Plata Island (Natural History, Jan., 1945, p. 22). In moist sheltered places grow *Coccoloba Ruiziana,* a small irregular tree. *Buettneria glabrescens* and *Carica paniculata* are shrubs characteristic of the zone from Santa Elena to Ancon. The *Carica* (pl. 4, fig. 6), with its pink flowers often on naked stalks before the leaves, is one of the few spectacular plants of the region. *Zinnia peruviana* is occasional in this zone, but nowhere as abundant



as in the Amotape region of Peru. In ravines the yellow-flowered *Thevetia peruviana* is conspicuous.

South of Ancon the savannas (pl. 2, fig. 6) are sandy and in these are formed small pools, some of them—as may be judged by the presence of water-lilies—of a somewhat permanent nature. In such pools grow *Azolla, Lemna, Heteranthera,* and a species of *Isoetes*. The Chanduy Hills (pl. 4, fig. 3, 4) are covered by an impenetrable tangle of arborescent cactus and trees of *Maytenus* and *Achatocarpus* interwoven with climbing cactus (*Hylocereus*), *Mimosa albida, Alternanthera dentata,* and *Plumbago scandens*.

The headlands at Punta Centinela (pl. 4, fig. 1) have a sparse vegetation. *Lantana Sprucei* and *Cissus cissioides* form flattened thickets on the sea cliffs. *Monnina chanduyensis,* the strong-scented *Porophyllum macrocephalum,* and *Verbesina Eggersii* occur as scattered plants in rocky places, and on the sandy beaches at tide level are *Sporobolus virginicus, Oxalis Cornelli, Talinum triangulare,* and *Cyperus confertus*.

This outer barrier of headlands continues, with occasional breaks, to Punta Ayangue, which marks the end of the dry zone. Shrubs on the outer rocks show the effects of southwest winds, being sheared to form a *krumholz*. A break in this outer barrier forms a long passage to the inner beach, where the vegetation is of a more tropical nature and coconut palms abound. On the beach are small clumps of the nicker-nut (*Caesalpinia Bonducella*).

At Manglaralto, a few miles to the north and directly under the influence of moisture condensation from the Cordillera de Colonche (pl. 2, fig. 1, 3), the strand vegetation is that of a region of moderate rainfall in the tropics. The manchineel tree (*Hippomane mancinella*) is occasional, the candle-nut tree (*Aleurites*) is abundant, and a yellow-flowered cotton (*Gossypium*) appears now and then, together with *Caesalpinia Bonducella, Cyperus ligularis, Cassia occidentalis,* and the purple-fruited *Scaevola Plumieri* as undergrowth. *Croton rivinaefolius* reaches its best development here. The hillsides exposed to the sea still show the effect of winds, the thickets of *Lantana Sprucei* being noticeably sheared, but the stream courses and other sheltered areas foster a tropical rain forest. The most prominent tree is a white-flowered composite (*Vernonia baccharoides*) which reaches a height of forty feet. The stream is bordered, in addition to large forest trees, by *Cecropia* and tall bamboos (*Guadua angustifolia*) (pl. 5, fig. 4). A pink-flowered *Heliconia* (probably *H. latispatha,* described from nearby Salango) is common locally in the forest. *Cestrum auriculatum,* with fragrant greenish flowers, is a common bush. Two species of *Jacquemontia* (*J. corymbulosa* and *J. nodiflora*) climb over bushes, among which are the fragrant blue-flowered *Cornutea odorata,* the widespread *Chiococca alba,* and *Myriocarpa stipitata*. Of climbing plants there are

Fig. 15. Distribution of *Cyperus elegans, Hippomane Mancinella, Ipomoea incarnata.*

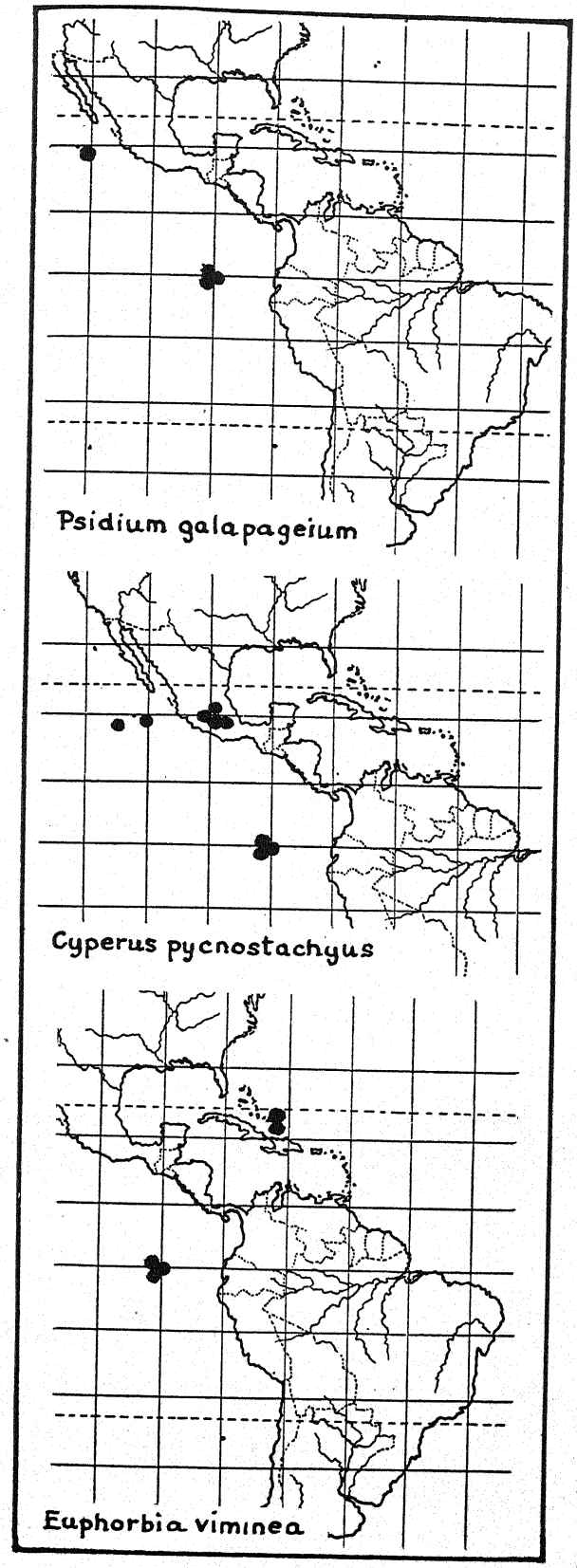


Fig. 16. Distribution of *Psidium galapageium, Cyperus pycnostachyus, Euphorbia viminea.*

also *Philodendron oligospermum, Smilax tuberculata, Dioclea Lehmanni,* and *Mikania micrantha.* Numerous epiphytes perch on moist tree-trunks, among them being *Pilea microphylla,* species of *Peperomia,* several bromeliads, and a few species of orchids, none of which were in flower during my visit. Ferns are scarce, and with the exception of *Polypodium balaonense,* they are all common tropical species. The chief industry of Manglaralto is the raising of vegetable ivory and of toquilla fiber. In addition, there are plantations of common tropical fruits, some of which have been abandoned and have grown up into jungle. In plantation openings will be found weedy plants which occur through the moist tropics, such as *Euphorbia graminea, Eryngium foetidum, Bouchea fluminensis, Hyptis mutabilis* and *H. pectinata, Ocimum micranthum, Justicia comata, Synedrella nodiflora,* and *Wedelia trilobata.* The affinity of the plants is with the wet coast of northern Ecuador and the lower slopes of the Andes, as at Bucay (75 miles west of Guayaquil) where I also saw *Cornutia odorata.* Southward these plants of the tropical rain forest appear on the damp eastern slopes of the Andes in Peru.

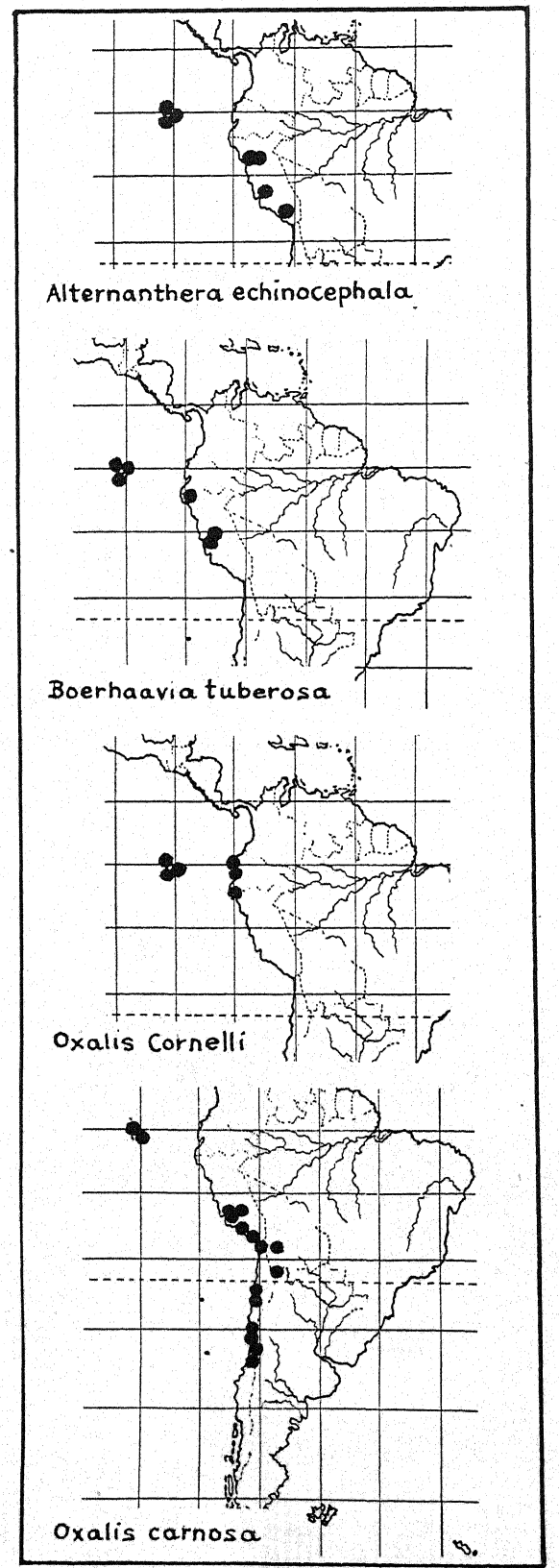
Eggers (1894) has given an excellent account of the flora of southwestern Ecuador (p. 275): The high mangroves of Puná Island reach 50 m. in height; in the sandy areas of Puná, they are smaller. With them are *Avicennia, Laguncularia,* and *Conocarpus,* and *Chrysodium* growing to a man's height. On the outer coast these are only at the mouths of rivers.

"The vegetation of the coast is psammophile, much like that of the West Indies, and the components and development are in general the same, except that the substratum of sand is less nutritive than coral debris. Air conditions (salt, etc.) are probably more important than the nature of the substratum.

"Coconut palms are as well developed at El Recreo as in the West Indies, also *Hippomane Mancinella, Paritium tiliacium,* and *Conocarpus erecta.* Here is lacking the West Indian *Coccoloba uvifera,* but in its place is *Cryptocarpus.* Among other shrubs are *Scaevola Plumieri* and *Guilandina Bonducella.* The trees are cut by the southwest winds just as in the Dutch West Indies. On the upper border of the sand are *Heliotropium curassavicum, Philoxerus vermiculatus, Sida ciliaris, Cyperus brunneus, Sporobolus littoralis* and *Canavalia obtusifolia,* which are all in the West Indies.

Among economic plants (p. 278) are the bamboo (*Guadua angustifolia*) and the *frutilla* (*Muntingia*). The trees (cocoa, coffee, and guava) grow to a large size, and the bamboo becomes 12 m. high and 25 cm. in diam. and is known as *caña brava.*" The split cane is sent in large quantities to Peru for boards for trimming the houses (cf. pl. 2, fig. 2 and pl. 4, fig. 5).

During the short period of rain, the dry zone



trees and bushes become leafy and bloom. In May Eggers found the woods wholly bare on Puná Island; in July at Manta there was no green leaf as far as the eye could reach. Humus formation in these woodlands is meager, because necessary moisture is lacking and dried leaves are soon converted to dust.

A word should be said about the vegetation of the inner coastal plain which occupies the vicinity of Guayaquil, the cocoa-producing area south to Machala, and the mangrove islands at the head of the Gulf of Guayaquil. Here, as Guppy (1906, p. 6) says, is probably the finest mangrove growth in the world. "The mangrove zone, marking a more or less abrupt transition from a region of drought and semi-sterility to one of humidity and rank tropical vegetation, begins about lat. 3° 30′ S, that is, in the vicinity of Tumbez, or perhaps nearer the boundary-line between Ecuador and Peru in lat. 3° 20′. Occupying the southern shore of the Gulf of Guayaquil it extends up the Guayas estuary to Guayaquil and rather beyond. But when we follow the coast of Ecuador northward from the island of Puná toward Santa Elena Point, we come upon one of the most remarkable phenomena presented on the west coast of South America. The dry region begins again and the mangroves disappear; and these conditions continue through about 2½ degrees of latitude until we reach the equator, when the mangrove zone soon recommences, and, as I infer, continues northward without a break to the coast of Central America.

"When we enter one of the numerous broad creeks that intersect the border of the mangrove-belt we soon find ourselves in the true mangrove forest, where prevail tall trees of *Rhizophora mangle* that rise to a height of 70 or 80 feet or more.[12] Gloomy as the depths of the swamp are, they acquire quite a funereal aspect, the branches of the trees being draped with pendant *Tillandsias*."

GEOGRAPHIC DISTRIBUTION OF THE GALAPAGOS—GULF OF GUAYAQUIL FLORA

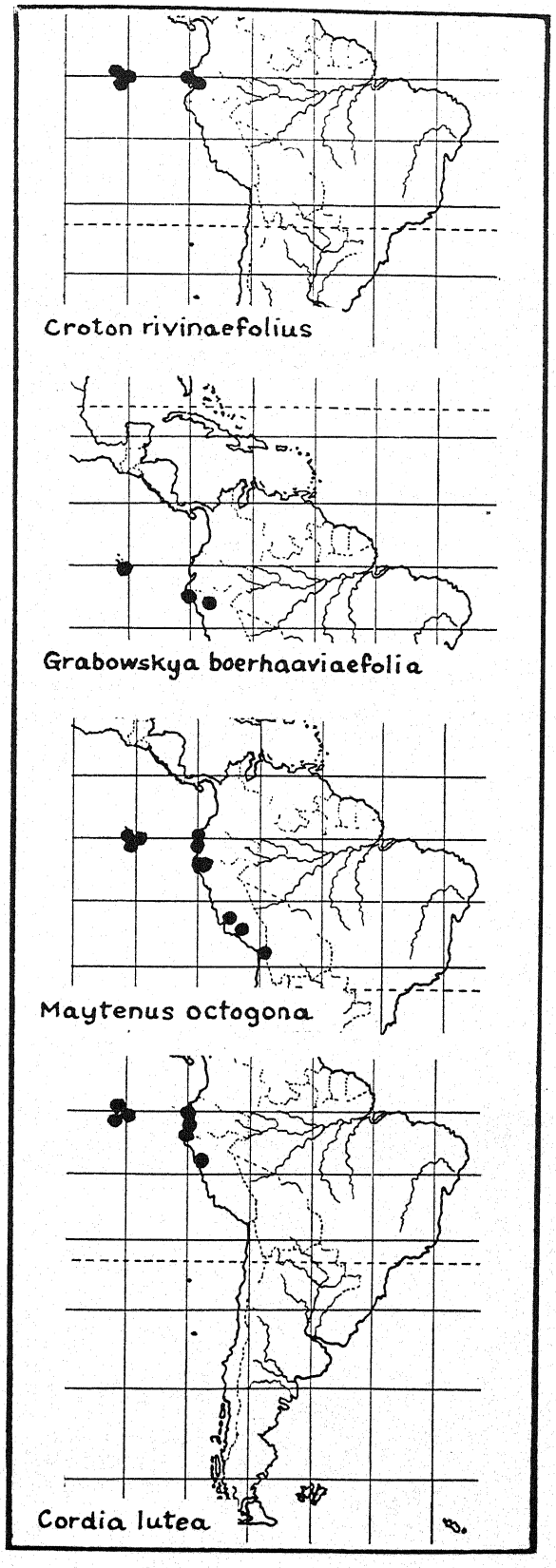
(Explanatory notes to accompany distribution maps follow this statistical treatment)

The plants of the Galapagos Islands and the adjacent coast fall into a series of groups from the point of view of geographic distribution, correlated with ecological conditions.

I. Rain forest.—No rain forest occurs, in my opinion, on the Galapagos Islands. On the continent the tropical rain forest extends unbroken from the Gulf of Panama to the Equator in Ecuador. South of the Equator it is represented along the coast only in a few isolated patches, the southernmost of which is at Manglaralto (1° 49′ S). From this re-

[12] In the Galapagos Islands, as at Academy Bay, on Indefatigable I., dwarf mangroves occur along the shore, and tall straight trees at the head of the inlet, a situation much the same as Guppy describes here (Svenson, 1930b).

Fig. 17. Distribution of *Alternanthera echinocephala, Boerhaavia tuberosa, Oxalis Cornelli, Oxalis carnosa.*



gion the zone of rain forest curves eastward to the lower slopes of the Andes in Ecuador, thence to the river valleys beyond the western Cordillera of Peru. At Manglaralto it is represented by such species as:

Cat. no. (see annotated list of plants in this paper).
59. *Philodendron oligospermum.*
61. *Aechmea pyramidalis.*
76. *Piper aduncum.*
77. *Piper medium.*
79. *Myriocarpa stipitata.*
81. *Pilea microphylla.*
100. *Celosia virgata.*
144. *Dioclea Lehmanni.*
164. *Monniera trifolia.*
244. *Cordia bifurcata.*
262. *Hyptis mutabilis.*
285. *Justicia comata.*
313. *Mikania micrantha.*

II. Wide-ranging species of the Galapagos Islands and the continent.—

A. Moist zone.
291. *Chiococca alba* (fig. 12).
—. *Doryopteris pedata* (fig. 12).
—. *Ionopsis utricularioides* (fig. 12).

B. Dry zone.
—. *Zanthoxylum Fagara* (fig. 13).
165. *Bursera graveolens* (fig. 13).
—. *Passiflora suberosa* (fig. 13).
223. *Vallesia glabra* (fig. 14).
227. *Evolvulus glaber* (fig. 14).

C. Littoral species.
—. *Scaevola Plumieri* (fig. 14).
49. *Cyperus elegans* (fig. 15).

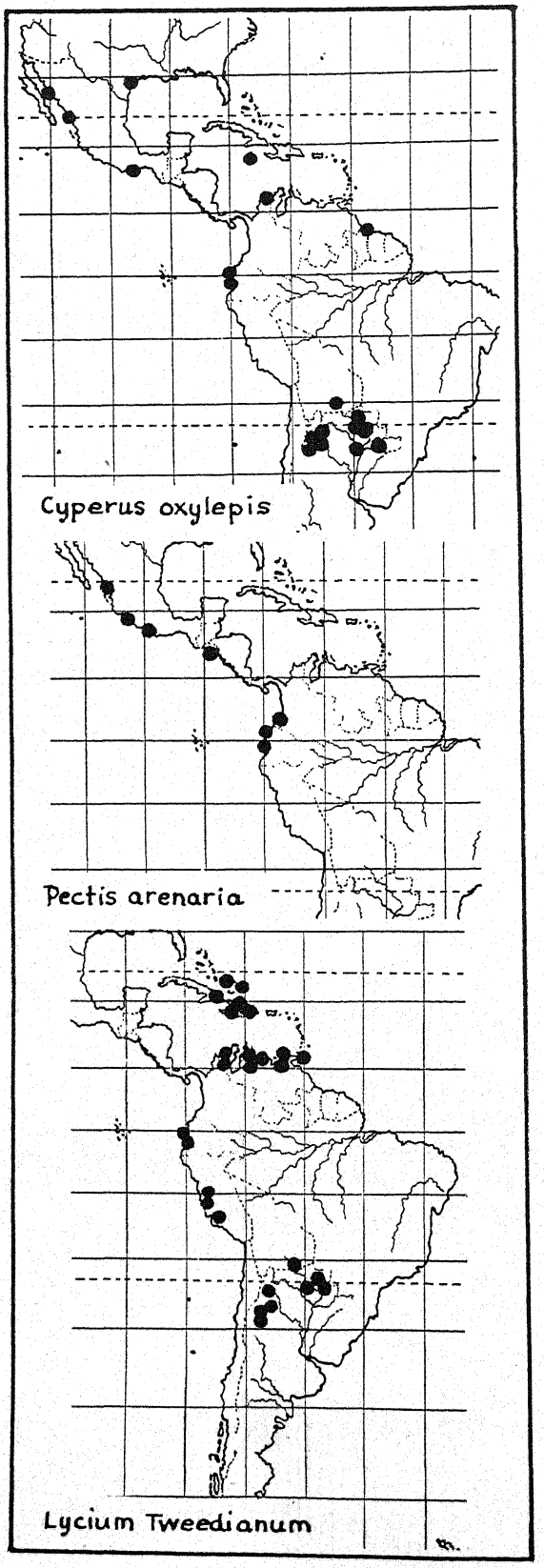
III. Galapagos endemics.—See Stewart (1911), Riley (1925), and Howell (1933–1941).

IV. Species restricted to the Galapagos Islands and Mexico.—
—. *Psidium galapageium* (fig. 16).
—. *Cyperus pycnostachyus* (fig. 16).

V. Species restricted to the Galapagos Islands and the West Indies.—
—. *Euphorbia viminea* (fig. 16).

VI. Species restricted to the Galapagos Islands and the adjacent coast of South America (often extending into the lower western slopes of the Peruvian Andes).—
9. *Polypodium balaonense.*
33. *Eriochloa pacifica.*
86. *Alternanthera echinocephala* (fig. 17).
88. *Alternanthera Helleri.*
91. *Alternanthera pubiflora.*
95. *Alternanthera vestita.*
104. *Iresine Edmonstonei.*
109. *Boerhaavia tuberosa* (fig. 17).
111. *Cryptocarpus pyriformis.*
122. *Boussingaultia filiformis.*
131. *Acacia macracantha.*
151. *Neptunia pubescens.*
157. *Stylosanthes sympodialis.*

Fig. 18. Distribution of *Croton rivinaefolius, Grabowskya boerhaaviaefolia, Maytenus octogona, Cordia lutea.*



161. *Oxalis Cornelli* (fig. 17).
—. *Oxalis carnosa* (fig. 17).
171. *Croton rivinaefolius* (fig. 18).
183. *Maytenus octogona* (fig. 18).
185. *Scutia spicata.*
192. *Malvastrum depressum.*
194. *Sida campestris.*
201. *Waltheria ovata.*
229. *Evolvulus simplex.*
245. *Cordia lutea* (fig. 18).
252. *Tournefortia psilostachya.*
256. *Clerodendron molle.*
268. *Cacabus prostratus.*
271. *Grabowskya boerhaaviaefolia* (fig. 18).
278. *Capraria peruviana.*
286. *Ruellia floribunda.*
301. *Luffa Astori.*
303. *Acanthospermum microcarpum.*

VII. Wide-ranging species of the Ecuadorean littoral.—Only a few have been selected. For the ecological similarities between this coast and certain West Indian islands, see the foregoing account by Eggers [p. 419].

53. *Cyperus oxylepis* (fig. 19).
272. *Lycium Tweedianum* (fig. 19).
315. *Pectis arenaria* (fig. 19).

VIII. Ecuador-Peru coastal endemics. — This list includes species of my collection, and could be greatly extended.

64. *Tillandsia latifolia.*
66. *Tillandsia straminea.*
70. *Hymenocallis quitoensis.*
83. *Psittacanthus ovatus.*
84. *Coccoloba Ruiziana.*
113. *Achatocarpus pubescens.*
118. *Portulaca Haughtii.*
125. *Capparis angulata.*
128. *Capparis ovalifolia.*
130. *Acacia Huarango.*
132. *Aeschynomene pluriarticulata.*
134. *Cassia corymbosa.*
154. *Pithecolobium candidum.*
166. *Monnina chanduyensis.*
168. *Acalypha subcastrata.*
182. *Loxopterygium Huasango.*
197. *Sida Weberbaueri.*
199. *Buettneria glabrescens.*
203. *Hybanthus circaeoides.*
211. *Cereus Cartwrightianus.*
224. *Calonyction Pavonii.*
226. *Cuscuta Haughtii.*
233. *Ipomoea crassifolia.*
241. *Jacquemontia secunda* (fig. 20).
258. *Lantana Sprucei.*
267. *Browallia elata.*
276. *Solanum cyathocalyx.*
287. *Ruellia pacifica.*
297. *Apodanthera biflora.*
299. *Cyclanthera leptostachya.*
311. *Isocarpha microcephala.*
322. *Spilanthes alba.*
324. *Verbesina Eggersii.*
328. *Zinnia peruviana.*

Fig. 19. Distribution of *Cyperus oxylepis, Pectis arenaria, Lycium Tweedianum.*

IX. Peruvian endemics.—This includes only species of my collecting. With the exception of *Hoffmannseggia viscosa*, they are confined to Piura and Tumbez, as in the map of *Eremocharis dissecta* (fig. 20).

34. *Eriochloa peruviana.*
82. *Psittacanthus linearis.*
90. *Alternanthera peruviana.*
109. *Boerhaavia verbenacea.*
148. *Hoffmannseggia viscosa* (fig. 20).
210. *Cactus Townsendii.*
218. *Eremocharis dissecta* (fig. 20).
228. *Evolvulus piurensis.*
249. *Heliotropium piurense.*
266. *Brachistus Haughtii.*
274. *Solanum amotapense.*
277. *Solanum talarense.*
282. *Proboscidia peruviana.*
310. *Heterospermum maritimum.*
318. *Piquieria peruviana.*
321. *Simsia Dombeyana.*

X. Chile-Peru coastal endemics.—(Of the same nature as these plants is *Coldenia paronychioides*, which extends to southern Ecuador. The list could be greatly extended and it contains no representatives of the Loma formation.)

94. *Alternanthera truxillensis.*
155. *Prosopis limensis.*
—. *Cereus macrostibas.*
232. *Ipomoea chilensis.*
243. *Coldenia paronychioides.*
279. *Galvesia fruticosa.*
309. *Encelia canescens* (fig. 20).

XI. Endemics of southern Ecuador coast.—To these could be added many species from other collections. The asterisks mark species of the inner moist zone.

12. *Marsilea ancylopoda.*
13. *Isoetes pacifica.*
40. *Pennisetum occidentale.*
61. **Aechmea pyramidalis.*
102. **Irenella chrysotricha.*
167. *Polygala alopecurus.*
211. **Hylocereus polyrhizus.*
239. **Jacquemontia corymbulosa.*
288. *Tetramerium nervosum.*
289. *Borreria Anderssonii.*
292. *Diodia incana.*

XII. Species extending inland to the Cajamarca region of Peru.—

130. *Acacia Huarango.*
204. *Carica paniculata.*
220. *Jacquinia pubescens.*
246. *Cordia polyantha.*
250. *Heliotropium rufipilum* (also Galapagos).
271. *Grabowskya boerhaaviaefolia* (also Galapagos).
286. *Ruellia floribunda* (also Galapagos).

XIII. Mediterranean (Europe-Africa) species.—

114. *Mollugo Cerviana* (also in Galapagos Islands, cf. Howell, 1933).
202. *Bergia capensis.*
298. *Cucumis dipsaceus.*

Explanatory notes for distribution maps

II A

Chiococca alba (L.) Hitchcock (fig. 12) (Cat. no. 291).—In the Galapagos Islands this somewhat climbing shrub has golden yellow flowers. At Academy Bay on the moist side of Indefatigable Island it has the greatest range of any species, extending from sea level to about 2000 ft., and it occurs also on the moister parts of Abingdon, Albemarle, Bindloe, Charles, Chatham, Duncan, James, and Narborough Islands. Though of wide distribution in the American tropics, *Chiococca alba* comes southward on the coast of Ecuador only as far as Manglaralto (which lies within the moist zone), and in Peru is found only in the trans-Andean region. The map has been compiled from Standley's citations in Field Mus. Bot., vol. 7 (1930–1931) and vol. 13, pt. 6 (1936), except for the Brazilian stations, which are from herbarium specimens. These stations could be greatly increased, especially from Brazil.

Doryopteris pedata (L.) Fée. Tryon, Contrib. Gray Herb. n.s. 143. 1942 (fig. 12).—This fern, which grows best on damp logs and rocks in moist regions of the Galapagos Islands, is known only from Abingdon, Albemarle, Charles, Chatham, Indefatigable, and James, i.e., the larger and moister islands. The distribution map is from Tryon (l.c.).

Ionopsis utricularioides (Sw.) Lindley (fig. 12). Epiphytic orchid with purple-striate white flowers, on Indefatigable Island at 400–800 ft. According to Stewart, it ascends to 1300 ft. on Charles Island, but does not reach above 1000 ft. on James. It is known also from Albemarle and Duncan Islands, always from the moist areas. According to Stehlé, Flore Descript. Antilles Françaises 1: 236. 1939, it is an epiphyte in Guadeloupe and Martinique on *Crescentia* and *Citrus* trees under cultivation, in a zone varying from moderate humidity to rather intense dryness. The map is from citations in Ames, Orchidaceae 5: 21–22. 1905.

II B

Zanthoxylum Fagara (L.) Sarg. (fig. 13).—A characteristic thorny tree or bush in the moist to dry parts of the Galapagos Archipelago. At Villamil, on Albemarle Island [at the base of a moist mountain slope corresponding to the Academy Bay region of Indefatigable Island], Stewart (l.c.) notes "bushes on lava near the coast, increasing in size with the elevation until they form small trees around 1300 ft.; above 1500 ft. they form bushes or low stunted trees. A few specimens were found on the rim of the crater at 3150 ft., and on the floor at 2750 ft." According to Sargent (N. Am. Silva 1: 73–74. 1891) the species is widely distributed from 29° N. in Florida to Brazil and Peru. Though abundant in the Bahamas, *Zanthoxylum Fagara* is lacking in a large part of the West Indies, and is not known to Porto Rico and the Virgin Islands.

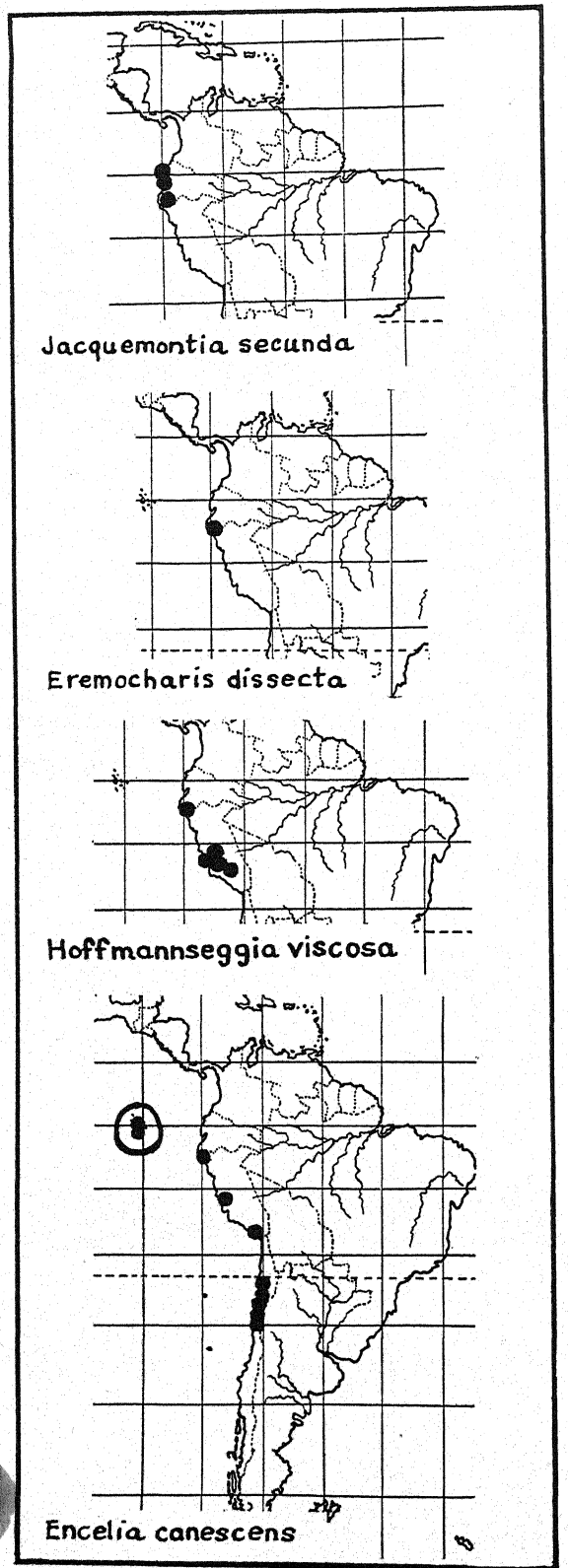


Fig. 20. Distribution of *Jacquemontia secunda, Eremocharis dissecta, Hoffmannseggia viscosa, Encelia canescens.*

Urban, Bot. Jahrb. 21: 577. 1896, cites it without further locality from Ecuador.

BURSERA GRAVEOLENS (HBK) Triana and Planch. (fig. 13) (Cat. no. 165).—The fragrant "palo santo" is, according to Stewart, "one of the most common trees in the dry and transition regions on the [Galapagos] Islands. It seldom attains a great height, usually having a broadly spreading crown and a short thick trunk." This species appears to be the only tree on the dry lavas of Tower Island. The distribution map is compiled from Engler in DC. Mon. 4: 49. 1883, but I have seen specimens only from South America.

PASSIFLORA SUBEROSA L. (fig. 13).—A species of wide distribution known from the moist to dry parts of Abingdon, Albemarle, Chatham, Duncan, Indefatigable, and James Islands. Map compiled from Killip, Field Mus. Bot. 19: 96. 1938. "Throughout the range of the species the occurrence of entire and deeply lobed leaves on the same individual is common. On the Galapagos Islands, for example, there are two variants; one (*P. linearilobа* Hook. f.) has very narrow leaf lobes, exactly matching part of the material . . . . from Florida; the other (*P. tridactyloides* Hook. f.) agrees closely with the Browne collection from Jamaica . . . ." (Killip, l.c.).

VALLESIA GLABRA (Cav.) Link. (fig. 14) (Cat. no. 223).—This species (from which *V. pubescens* is not distinguishable) occupies dry coastal areas in the Galapagos. The map was made chiefly from specimens in the Herbarium of the New York Botanical Garden. The only collection seen from Colombia is *Killip and Hazen* 11,075, from a desert river-bottom in the Dagua Valley. Distribution in the West Indies represents *V. antillana* Woodson, a related but distinct species.

EVOLVULUS GLABER Spreng. (fig. 14) (Cat. no. 227).—Map compiled from citations by Van Oostroom, Bot. Mus. Utrecht Med. no. 14: 97–99. 1934.

II C

SCAEVOLA PLUMIERI (L.) Vahl. (fig. 14).—The buoyant stony fruits of this plant may float unharmed in sea water for four or five months, according to the extensive investigations of Guppy (1917, pp. 227–236). The purplish-black fleshy fruits attract birds, but long-distance transportation has been largely by ocean currents. It is of sporadic occurrence in the New World, and known on the Pacific Coast only from the Revillagigedo Islands, the Galapagos Islands, and the coast of Ecuador. Eggers reported it as one of the characteristic strand plants of western Ecuador, and I found it in fine fruiting condition on the sandy beaches at Manglaralto in March, 1941. It seems to be confined to a relatively moist zone, and not on the dry coast south of Manglaralto. The genus is almost entirely Australian, but *S. Plumieri* is an exception, being one of the outstanding strand plants on the west coast of Africa from Senegal to Benguela, and on the east coast from Somaliland southward to the

Cape of Good Hope. It extends eastward to India, Ceylon, and Mauritius. The map was prepared chiefly from localities cited by Krause in Pflanzenreich IV^{277}: 121. 1912.

CYPERUS ELEGANS L. (*C. rubiginosus* Hook. f.) (fig. 15) (Cat. no. 49).—On the Galapagos Islands the spikelet scales of *C. rubiginosus* vary from dark red to pale brown. Elsewhere, as Kuekenthal (op. cit., p. 164) states, they are frequently purplish-tinged in *C. elegans*. This species seems centered about the Caribbean area, chiefly near the coast, but is known from western Texas, and perhaps from New Mexico. (O'Neill in Tidestrom and Kittell, Flora of Arizona and New Mexico. 766. 1941). On the Galapagos Islands it occurs in sandy places just above tide level, on most of the islands.

HIPPOMANE MANCINELLA L. (fig. 15).—A tree with poisonous juice, which in the Galapagos sometimes extends inland. Map from citations by Pax, Pflanzenr. IV^{147}, pt. v: 162. 1912; Standley, Trees and Shrubs of Mexico 629. 1923; and Bentham, Bot. Sulphur 60. 1844.

IV

PSIDIUM GALAPAGEIUM Hook. f. (fig. 16).—Johnston (1931, p. 80) has referred a collection from Socorro Island to this species of guava, which was previously considered as endemic to the Galapagos Islands.

CYPERUS PYCNOSTACHYUS (HBK) Kunth. *C. duripes* Johnston, Proc. Cal. Acad. ser. 4, 20: 54. 1931. *C. Anderssonii* Boeckl., Linnaea 36: 388. 1870 (fig. 16).—In Calif. Acad. ser. 4, 22: 192. 1939, I stated that *C. Anderssonii* was probably a reduced derivative of *C. duripes* of the Revillagigedo Islands, and Kuekenthal, Pflanzenr. IV^{20}: 440. 1936, has included *Anthony* 400 from Socorro Island under *C. pycnostachyus*, a species of central Mexico. Some of the Galapagos specimens, such as *Howell* 10, 121 from Tower Island, are about as large as the Mexican material, but in general the plants from the Galapagos Islands (*C. Anderssonii*) are reduced in size, showing the dwarfing characteristic of the islands. From this point of view it would be logical to derive the Galapagos material from Mexican. Some of the Galapagos material has spikelets 2- (rarely 3-) fruited, and such specimens do not differ essentially from the Mexican material. The section *Thunbergianae*, which includes *C. pycnostachyus*, Kuekenthal considers (p. 403) as having scales spreading at the apex, while those of *C. Anderssonii* belong to the section *Turgidulae* which has scales appressed. All specimens appear to me to have scales equally appressed. In all these plants the rachilla is prominently winged, and I cannot see that more than a single species is represented.

V

EUPHORBIA VIMINEA Hook. f. Trans. Linn. Soc. 20: 184. 1847. *E. vaginulata* Griseb. Fl. Br. W. Ind. 52. 1860 (fig. 16).—This species seems to be the only plant confined to the West Indies and the Galapagos. Various forms were described by Robinson and Greenman (1895, p. 138–139) as illustrating variation from island to island. My paper on the Galapagos Islands (1935, p. 240 and pl. 2) pointed out that much of this variation was due to differences between juvenile and adult foliage. The variation is not more than might be expected in any group of islands with variable climatic conditions, and collections from the Bahamas (Field Museum; hb. Brooklyn Botanic Garden) show approximately the same variation as in Galapagos material. Guppy (1917, pp. 280–289) knew the plant from the Turks Islands in the Bahamas, and (p. 285) describes the "burnt bush" as the most conspicuous of the Euphorbiaceae in the scrub flora of Grand Turk. It is accompanied by *Lantana involucrata* and *Borreria thymifolia*. He comments as follows: "The scrub vegetation of the sandy plains and of the gentler hill-slopes in the interior of the island extends to the summit of the hills when the surface is sandy."

Millspaugh (1906, p. 165) notes that *Euphorbia vaginulata* grows in great profusion on Grand Turk Island, and that the Inagua specimens apparently differ from those of Grand Turk and Little Inagua on account of their longer leaves and darker bark. Britton and Millspaugh (1920, p. 240) extend the range to Watling's and Castle Island.

In view of the identity of the Galapagos and Bahama *Euphoribia*, it seemed important to compare the six endemic species of *Borreria* (Rubiaceae) in the Bahamas, in so far as possible, with the fourteen endemic species of the Galapagos Islands listed by Stewart. The Galapagos species differ markedly from those of the Bahamas in the very short corolla tube, soft texture of capsules, and axillary inflorescence. Their relationship is obscure.

Guppy (p. 271) ". . . . would suggest with some diffidence that the climatic régime which now prevails on the sea-borders of North Chile and Peru once existed in the Bahama region. Just as the cold waters of the Humboldt or Peruvian Current determines the relative sterility of that continental seaborder . . . . so in the past a cold current from the north swept along the northern shores of the Bahamas and cut off the moisture carried by the northeast trade-winds. From this point of view the aeolian formation of the Bahamas represents the New World's response in ancient times to the influences producing arid sea-borders in warm latitudes (cold currents and drying winds), but in a region of calcareous sands and under insular conditions, and association of circumstances not repeated on the same scale in any other part of the globe."

(P. 272). "What, we may also ask, would be the behavior of the Arctic Current if the great Equatorial Current, from which the Gulf Stream takes its rise, flowed in mass, as it probably once did, across the site of the present Panama Isthmus into the Pacific? The effect of the emergence of this isthmus was the birth of the Gulf Stream, and the effect of the Gulf Stream was a dissipation to a

large extent in tropical latitudes of the cold current from the north, so that the Bahamian region would no longer come under its influence. Before the change we had the same association of aridity with a cold current that exists on the sea-border of Peru and also on the sea-borders of South California and North Mexico, where the moisture-laden winds, by passing over the cool waters of the Californian Current, become drying winds when striking the continent, and produce the aridity of its sea-border."

This paragraph I leave without further comment. The pirate, Woodes Rogers, was governor of the Bahamas, but long after his voyage (1708) to the Galapagos Islands; introduction into the Galapagos, or vice versa, of *Euphorbia vininca,* may more probably be due to the whaling industry.

VI

ALTERNANTHERA ECHINOCEPHALA (Hook. f.) Christophersen (fig. 17) (Cat. no. 86).—This is not a member of the Loma flora of the Peruvian coast, but of the shrubby inland vegetation. Suessenguth cites this species (as *A. tubulosa*) from Oroya, Dept. Junin (*Raimondi* 12,569), and from as high as 1800 m. on rocky western slopes of the Andes, near Pisco (*Weberbauer* 5405). It is one of the most abundant species of *Alternanthera* in the Galapagos, occurring on practically all of the islands, but there is not much variation except in width of leaves. It runs from sea level to 2100 ft. on Albemarle Island and on James to over 1000 ft.

OXALIS CARNOSA Molina, Sagg. Chile, ed. 2, 288. 1810 (fig. 17).—Rather sterile places in the transition and moist regions of Abingdon, Charles, and Duncan Islands, the roots finding lodgment in small crevices of lava (Stewart, 1911, p. 79). Common on the coast between Lima and Santiago, and extending inland in Peru and Bolivia. The map is based chiefly on citations by Knuth, Pflanzenr. IV^{130}: 186. 1930.

VII

CYPERUS OXYLEPIS Nees. (fig. 19) (Cat. no. 53).—A plant chiefly of the saline regions of Argentina. The map is based on data in Kuekenthal, Pflanzenr. IV^{20}: 167. 1936; Barros, Anales Mus. Argent. Nat. 39: 313. 1938; O'Neill in Rhodora 40: 358. 1938; and McGivney, Catholic Univ. Biol. Ser. no. 26: 28. 1938.

LYCIUM TWEEDIANUM Griseb. (fig. 19) (Cat. no. 272).—A spinescent shrub described originally from the arid saline area of western Argentina. The map has been compiled from citations by C. L. Hitchcock, Annals Missouri Bot. Gard. 19: 221. 1932. Perhaps *L. minimum* of the Galapagos Islands should be included in this species.

X

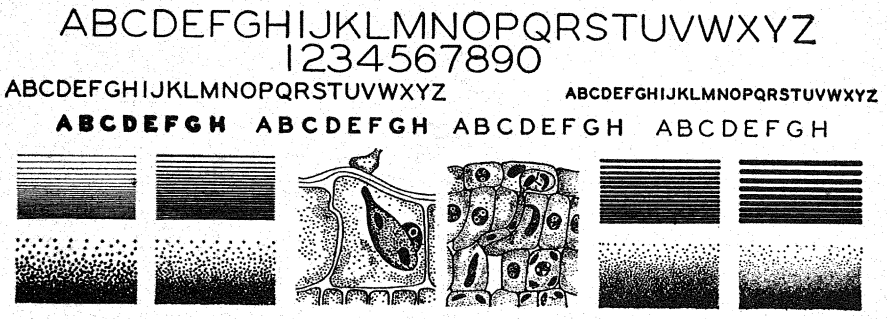
ENCELIA CANESCENS Lam. and *E. hispida* Anders. [Galapagos Islands] (fig. 20) (Cat. no. 309).—The map has been compiled from citations by Blake, Proc. Am. Acad. 49: 369, 370. 1913, and I. M. Johnston, Contrib. Gray Herb. no. 85: 126. 1929. The mainland plant (*E. canescens*) has soft pubescence; the Galapagos plant is "hispid-canescent." Andersson's material was determined by Steetz who thought it very close to the Chilean plant, differing from the phase *E. oblongifolia* in several characters of leaves and inflorescence.

EXPLORATION

The bibliography of exploration in the Galapagos Islands has been adequately cited by Robinson (1902) and by Stewart (1911). For the coast of Ecuador, a list of botanical collectors and their itineraries has been prepared by Diels (1937, pp. 43–55). The most important botanical collectors in coastal Ecuador are: Joseph Jussieu (1735), a member of La Condamine's expedition; Tafalla (1800?) who accompanied Ruiz and Pavon; Humboldt and Bonpland (1802–1803); Jameson, in Guayaquil (1822–1826); Hinds (1838) and Hartweg (1841–1843), whose collections were written up by Bentham (1839–1846); Spruce (1862) and Eggers (1891–1892). Later authors are mentioned in the "Catalogue of Plants." Herrera (1939) has provided an excellent account of botanical explorers in Peru (1939, pp. 3–90).

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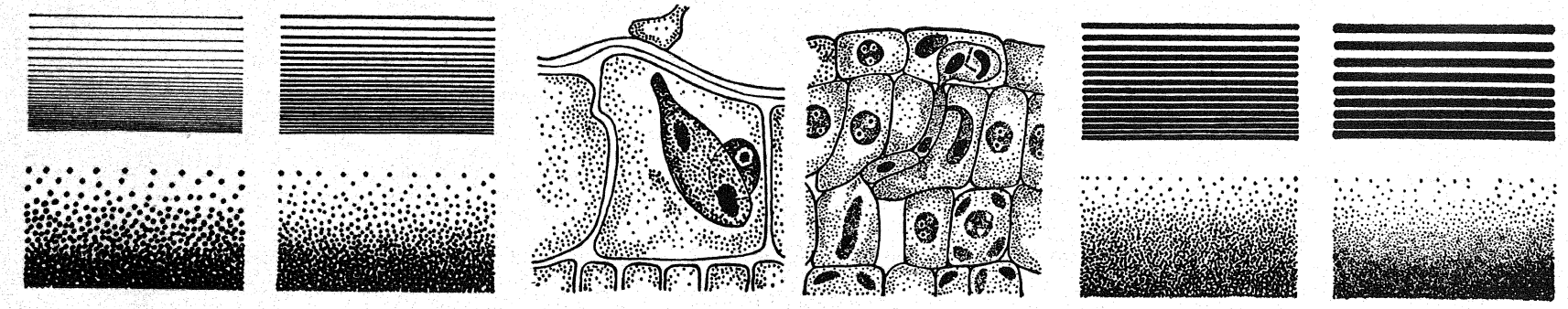
Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to ¼. Middle—Reduction to ½. Bottom—Original size.

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Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

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VEGETATION OF THE COAST OF ECUADOR AND PERU AND ITS RELATION TO THAT OF THE GALAPAGOS ISLANDS. II. CATALOGUE OF PLANTS

Henry K. Svenson

(Collections cited as "H.&S" are by *Haught & Svenson*)

POLYPODIACEAE

1. ADIANTUM CONCINNUM Humb. & Bonpl. ex Willd. Sp. Pl. 5: 451. 1810; Maxon in Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 424. 1926. A slender maiden-hair in plantations; Manglaralto (11,475). Widely distributed from West Indies southward, and known from Galapagos Islands.

2. A. TETRAPHYLLUM Humb. & Bonpl. ex Willd. Sp. Pl. 5: 441. 1810; Maxon (op. cit., p. 423). A rough-pubescent fern in plantations, Manglaralto (11,480). West Indies and southward; questionably from the Galapagos Islands.

3. ASPLENIUM AURITUM Sw. in Schrad. Jour. 1800[2]: 52. 1801; Maxon (op. cit., p. 450). Epiphyte, Manglaralto (11,482). Abundant fern in tropical America; Galapagos Islands.

4. ASPLENIUM aff. POLOENSE Rosenst. in Fedde, Rep. Spec. Nov. 12: 469. 1913; Svenson, Bull. Torr. Bot. Club 65: 311. 1938. Terrestrial, in dense shade, Manglaralto (11,473). Larger than my collection of *A. poloense* from the Galapagos.

5. BLECHNUM OCCIDENTALE L. Sp. Pl. 1077. 1753; Maxon (op. cit., p. 456). Terrestrial fern in plantations, Manglaralto (11,481). From Florida southward, and in the Galapagos Islands.

6. DRYOPTERIS PATENS (Sw.) Kuntze, Rev. Gen. Pl. 2: 813. 1891; Maxon (op. cit., p. 471). *Polypodium patens* Sw. Prod. 133. 1788. Shaded ravine, Manglaralto (11,470). From Florida southward; Galapagos Islands.

7. D. POITEANA (Bory) Urban, Symb. Ant. 4: 20. 1903; Maxon (op. cit., p. 472). *Lastrea Poiteana* Bory, Dict. Class. 9: 233. 1826. Plantation, Manglaralto (11,487); similar to Galapagos specimens. West Indies; and from Guatemala to Peru and Brazil.

8. D. SUBTETRAGONUM (Link) Maxon in Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 473. 1926. *Dryopteris tetragona* Urban, Symb. Ant. 4: 20. 1903; not Kuntze, 1891. Shaded ravine, Manglaralto (11,489). From Florida southward; Galapagos Islands.

9. POLYPODIUM BALAONENSE Hieron. in Engler, Bot. Jahrb. 34: 529. 1904. Abundant epiphyte, Manglaralto (11,440). Described from Balao, Ecuador, it is a variant of *P. lepidopteris* with scales silvery, and not red as in Galapagos specimens. *P. lepidopteris,* originally from St. Catherine, Brazil, extends southward from the West Indies in variable forms (cf. Christensen, Mon. Dryop. 1: 261. 1913).

10. PTERIS aff. QUADRIAURITA Retz. Obs. Bot. 6: 38. 1791; Maxon (op. cit., p. 434). Plantations, Manglaralto (11,474). Non-fruiting, but evidently to be allied to this widespread species.

SALVINIACEAE

11. AZOLLA MICROPHYLLA Kaulfuss, Enum. Fil. 273. 1824; Svenson, Am. Fern Jour. 34: 82, pl. 6, f. 4; pl. 8, f. 3. 1944. A water-fern on surface of pools, west base of Chanduy Hills (11,025), and as in Galapagos Islands, accompanied by fruiting plants of *Lemna minima.*

MARSILEACEAE

12. MARSILEA ANCYLOPODA A. Br. Monatsber., Akad. Berl. 1863: 434. 1864; Baker, Jour. Bot. 24: 282. 1886. Abundant in ponds, Salinas (11,152). This endemic species, the solitary tomentose sporocarps borne on deflexed pedicels, is otherwise known only from Guayaquil (*Jameson* 394) and Chanduy (*Spruce*). It is probably closest to the Paraguayan *M. Hickenii.*

ISOETACEAE

13. ISOETES PACIFICA Svenson, Am. Fern Jour. 34: 123, pl. 10. 1944. In shallow pools at west base of Chanduy Hills (11,002). Endemic.

SELAGINELLACEAE

14. SELAGINELLA ERYTHROPUS Spring, Mon. 2: 155. 1849; A. Braun in Triana & Planchon, Prod. Fl. Nov. Granat. pt. 2: 364. 1865. Ravines, in dense shade, Manglaralto (11,472, det. Morton). Brazil; Colombia to Chile.

15. S. KUNZEANA A. Br. op. cit., p. 380. Stream banks, Manglaralto (11,471, det. Morton). Andes, from Colombia to Peru.

GRAMINEAE

(Cf. Hitchcock, Contrib. U.S. Nat. Herb. [Grasses of Ecuador, Peru and Bolivia] 24: 291–556. 1927; and Standley, in Macbride [Fl. Peru] Field Mus. Bot. 13, pt. 1: 1–320. 1926).

16. ANTHEPHORA HERMAPHRODITA (L.) Kuntze, Rev. Gen. Pl. 759. 1891; Hitchcock, p. 407. A common annual grass, with urn-shaped scales in a close spike, on rocks and open soil, Puntilla, Salinas (11,110 & 11,090); Punta Centinela (11,272); Punta Carnera (11,297); Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,548); also Chanduy (*Spruce*) and Piura (*Haught*). An abundant weed in tropical America.

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17. ARISTIDA ADSCENSIONIS L. Sp. Pl. 82. 1753; Hitchcock, p. 403. This species together with *Chloris virgata* are the chief forage grasses in the dry coastal area of Ecuador, and Haught (cf. Standley, p. 183) states that it is "probably the most abundant annual grass of northwestern Peru." Puntilla, Salinas (11,094, 11,325, & 11,121); Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,591). Described originally from Ascension Island. Common in dry ground throughout the tropics.

18. BOUTELOUA ARISTIDOIDES (HBK.) Griseb., Fl. Brit. W. Ind. 537. 1864; Hitchcock, p. 417. An inconspicuous decumbent grass, occasional in sandy fields and dunes, Salinas (11,309) and river gravels, Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,626). Collected in Piura by Weberbauer (Standley, p. 194) but not recorded from Ecuador. Southern United States to Argentina.

19. B. DISTICHA (HBK.) Benth., Jour. Linn. Soc. Bot. 19: 105. 1881; Hitchcock, p. 417. Abundant in open sandy fields and dry hills, Salinas (11,075, 11,132 & 11,091); Cerro Prieto, Piura, Peru (H&S 11,552a). Ecuador and northern Peru; introduced into Cuba. Chanduy (*Spruce*). Hitchcock, p. 418, says that *B. pilosa,* known from Guayaquil and ranging from Central America to northern Peru, is probably a form of *B. disticha.* It was originally described from the Galapagos Islands where the specimens have smaller, nearly awnless spikelets, due to awn reduction in the sterile floret. Length of the awn varies in continental *B. disticha* and also color of spikelets, those of 11,075 being purplish when mature instead of the usual green. Number of spikelets in the inflorescence also seems of minor importance.

20. CENCHRUS PILOSUS HBK. Nov. Gen. & Sp. 1: 116. pl. 36. 1816; Hitchcock, p. 489. An erect annual bur-grass with bristles antrorsely scabrous. Headlands and dunes north of Punta Carnera (11,298); Puntilla, Salinas (11,092); Punta Centinela (11,269); river gravels, Quebrada Ancha, near La Brea, Peru (H&S 11,564); Chanduy (*Spruce*). Hitchcock says (p. 489): "The Chanduy specimen is more pilose on the inner spines than most of the specimens from Central America." None of the numerous specimens collected by me is decumbent or rooting at the nodes, a usual character mentioned by Hitchcock. Areschoug (1910, p. 117) states that specimens collected by Andersson on Puna Island do not agree well with the HBK. plate (drawn from a young specimen), the Puná plant being more robust with extended spike and less pilose involucre. Open ground in cultivated soil from Mexico to Ecuador. Originally described from Venezuela.

21. CHLORIS RADIATA (L.) Sw., Prodr. 26. 1788; Hitchcock, p. 415. On seashore, Salinas (11,065). A common weed from the West Indies, Mexico, and Paraguay.

22. C. RUPESTRIS (Ridley) Hitchcock, Man. Grasses W. I. [U.S. Dept. Agric. Misc. Publ. no. 243]: 126. 1936. *Gymnopogon rupestris* Ridley, Jour. Linn. Soc. 27: 73. 1890. *C. anisopoda* Rob. Proc. Amer. Acad. 38: 118. 1902. *C. angustiflora* Aresch. Eugenies Resa Bot. 118. 1910; Fedde, Rep. Spec. Nov. 10: 300. 1912; Hitchcock, p. 415.

An inconspicuous slender species in rocky ground, Chanduy Hills (11,039); Puntilla, Salinas (11,324); base of Cerro Prieto, Piura, Peru (H&S 11,638); Quebrada Ancha, near La Brea, Peru (H&S 11,639). It was collected by Hitchcock between Guayaquil and Salinas, by Spruce at Chanduy, and by Andersson on Puna Island. West Indies to Brazil and Ecuador; Galapagos Islands.

23. C. VIRGATA Sw., Fl. Ind. Occ. 1: 203. 1797; Hitchcock, p. 414. The most abundant grass in the dry coastal region of Ecuador, in fields and on exposed seacliffs, Salinas (11,065a); Puntilla, Salinas (11,323, 11,095, 11,117). Open sterile ground from the West Indies to Argentina.

24. COTTEA PAPPOPHOROIDES Kunth, Rev. Gram. 1: 84. 1829; Hitchcock, p. 351. A low perennial with purplish stem and awned purple spikelets in a panicle, on rocky slopes near base of Cerro Prieto, Piura, Peru (H&S 11,635, det. Swallen). Collected by Spruce at Chanduy. Dry plains, southwestern United States and Mexico; Ecuador to Argentina.

25. DACTYLOCTENIUM AEGYPTIUM (L.) Richt. Plant. Europ. 1: 68. 1870; Hitchcock, p. 411. Punta Centinela (11,273); Salinas (11,069); Puntilla, Salinas (11,082 & 11,084); Quebrada Mogollon, Amotape Hills, Peru (H&S 11,640). Common in waste places, in all warm countries. On the Puntilla at Salinas this species grows exposed to ocean spray as dwarf plants 6–10 cm. high with the inflorescence reduced to 2 spikes. It is similar in appearance to the 1-spiked form on Charles Island in the Galapagos, such a reduction of inflorescence being probably due to the severe climatic conditions in both areas.

26. ECHINOCHLOA COLONUM (L.) Link, Hort. Berol. 2: 209. 1833; Hitchcock, p. 476. Frequent at the borders of pools, between Ancon and Santa Elena (11,177); south of Ancon (11,430); south of Engabao (11,023). A widely-distributed weed in both hemispheres.

27. E. CRUS-GALLI (L.) Beauv., Agrost. 53, 161. 1812. Borders of pools south of Engabao (11,031). Inflorescence varies from green to brown, with spikes erect, spikelets hispid, and awns only 2–3 mm. long. A common weed in both hemispheres. The typical plant, as treated by Hitchcock (Manual of the Grasses of the West Indies), does not seem to be reported from Ecuador.

28. ERAGROSTIS AMABILIS (L.) Wight & Arn. in Hook. & Arn. Bot. Beechey Voy. 251. 1841; Hitchcock, p. 336. A weedy plant with small-flowered elongate panicles, in sandy soil, Salinas (11,097). The closely related *E. viscosa* was collected by Spruce at Chanduy, and by Hitchcock east of Salinas. Open ground in warm regions of both hemispheres.

29. E. CILIANENSIS (All.) Link ex Vign. Lut., Malpighia 18: 386. 1904; Hitchcock, p. 337. Open

soil, Puntilla, Salinas (11,116 & 11,322). A European species common in waste places in the New World.

30. E. CILIARIS (L.) R.Br. in Tuckey, Narr. Exp. Congo App. 478. 1818; Hitchcock, p. 336. An abundant species in open soil of headlands and dunes, characterized by narrow spike-like panicles. Puntilla, Salinas (11,115, 11,134, 11,321); dunes, Salinas (11,191); Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,625). This widespread plant, originally described from Jamaica, was collected at Chanduy by Spruce.

31. E. MEXICANA (Hornem.) Link, Hort. Berol. 1: 190. 1827. *Poa mexicana* Hornem., Hortus Hafn. 2: 953. 1815; Hitchcock, p. 339. Close in appearance to *E. pilosa* from which it differs in having larger spikelets. Cerro Prieto, Piura, Peru (H&S 11,636). Fields and waste places, southwestern United States to Chile.

32. E. PILOSA (L.) Beauv., Ess. Agrost. 162. 1812; Hitchcock, p. 339. Margins of pools between La Liberatad and Santa Elena (11,424); hills north of Punta Carnera (11,303); low grounds, Salinas (11,286); Chanduy (*Spruce*). Warm and temperate regions of both hemispheres; originally described from Italy.

33. ERIOCHLOA PACIFICA Mez in Engler, Bot. Jahrb. 56. Beibl. 125: 11. 1921; Hitchcock, p. 429. Waste banks by the sea, Salinas (11,067); low hills north of Punta Carnera (11,305); low grounds north of Ancon (11,431). This annual, originally described from Puná Island (*Eggers* 14,772), often reaches a height of 2 ft. or more, and forms large patches with conspicuous pubescent, white or purple-tinged inflorescence. Chanduy (*Spruce*), Guayaquil (*Mille*), Puná Island (*Andersson* 29); Peru (*Weberbauer* 5289), and Galapagos Islands (according to Hitchcock, l. c.). Introduced into Queensland, Australia.

34. E. PERUVIANA Mez in Engler, Bot. Jahrb. 56. Biebl. 125: 12. 1921; Hitchcock, p. 429. A decumbent annual in river gravels, Quebrada Ancha, near La Brea, Peru (H&S 11,560). Known previously only from *Weberbauer* 5952, collected between Piura and Hacienda Nomala.

35. LEPTOCHLOA FILIFORMIS (Lam.) Beauv. Ess. Agrost. 71, 166. 1812; Hitchcock, p. 409. Low ground between Ancon and Santa Elena (11,183); south of Ancon (11,433); Chanduy (*Spruce*). A widespread species with slender racemes, ranging from southern United States to Argentina.

36. PANICUM FASCICULATUM Sw. Prodr. 22. 1788; Hitchcock, p. 458. A weedy plant on open headlands of the Puntilla, Salinas (11,093 & 11,331); low ground between Ancon and Santa Elena (11,180); Chanduy (*Spruce*). Southern Florida and Texas to Ecuador and Brazil and in Galapagos Islands.

37. P. MOLLE Sw. Prodr. 22. 1788. An annual resembling *P. fasciculatum*, but pubescent. Damp thickets, Chanduy Hills (11,014, det. Swallen). West Indies and Mexico to Argentina, unknown previously from Ecuador except in the Galapagos Islands (Chatham I., *Chiercha*).

38. PASPALUM RACEMOSUM Lam., Tabl. Encycl. 1: 176. 1791; Hitchcock, p. 439. In thickets or on rocky slopes, frequently with a reddish inflorescence. Cerro Prieto, Piura, Peru (H&S 11,637, det. Swallen). Cited by Hitchcock from "recently disturbed soils, fields, waste places, and roadsides, spreading or clambering among brush, Colombia to Peru."

39. P. VAGINATUM Sw. Prodr. 21. 1788; Hitchcock, p. 443. A single clump in sand, Salinas (11,435). Common on seacoasts in tropics of both hemispheres; Balao (*Eggers* 14,602), and cited from Guayaquil by Areschoug.

40. PENNISETUM OCCIDENTALE Chase, Contrib. U. S. Nat. Herb. 24, pt. 8: 483. 1927. Low ground north of Santa Elena (11,256). Known only from Ecuador: Chanduy (*Spruce* 6426) to Guayaquil, where the type was collected by Hitchcock. In the loamy black soil back of the coast it forms tremendous stands, up to 2 meters in height.

41. SETARIA SETOSA (Sw.) Beauv., Ess. Agrost. 51, 178. 1812. Damp woods, Chanduy Hills (11,007, det. Swallen). This species is not included by Hitchcock in the "Grasses of Ecuador, Peru and Bolivia" (which deals with the mainland), but is abundant in the Galapagos Islands. Dry or rocky woods from West Indies to Brazil.

42. SPOROBOLUS PYRAMIDATUS (Lam.) Hitchcock, U.S. Dept. Agric. Misc. Publ. no. 243: 84. 1936. *Agrostis pyramidata* Lam. Tabl. Encycl. 1: 161. 1791. *S. argutus* Kunth, Rév. Gram. 1: Sup. XVII. 1830; Hitchcock, p. 392.

Abundant on exposed sea cliffs, Puntilla, Salinas (11,118); dry, sandy border of salt marsh, Salinas (11,311); dunes, Punta Centinela (11,275); Chanduy (*Spruce*); river gravels, Quebrada Mogollon, Piura, Peru (H&S 11,541). In exposed places the panicle remains contracted and often does not emerge from the sheaf-like upper leaf. Cited by Hitchcock from "open sandy or alkaline soil, southwestern United States to Argentina," but strangely, not in the Galapagos Islands.

43. S. VIRGINICUS (L.) Kunth, Rév. Gram. 1: 67. 1829; Hitchcock, p. 392. A perennial with extensively creeping wiry rootstocks, on dunes, Punta Centinela (11,274) and borders of salt pits, Salinas (11,330); Chanduy (*Spruce*). Virginia to Brazil and Chile.

44. TRAGUS BERTERONIANUS Schultes, Mant. 2: 205. 1824. *Nazia aliena* (Spreng.) Scribn., U.S. Dept. Agric. Div. Agrost. Bull. 17: 28, f. 324. 1899; Hitchcock, p. 407. A low annual with burr-like spikelets, in dry open ground, Puntilla, Salinas (11,120); Quebrada Mogollon, Piura, Peru (H&S 11,535). Ranges, according to Hitchcock, Man. Grasses W.I., p. 101, 1936, from southwestern United States and the West Indies to Brazil. It was collected at Chanduy by Spruce, but is unknown from the Galapagos Islands.

CYPERACEAE

45. CYPERUS ALBOMARGINATUS Mart. & Schrad. ex Nees in Mart. Fl. Bras. 2, pt. 1: 9. 1842; Kuekenthal, Pflanzenr. IV[20]: 359, fig. 42 E–H. 1936. Tall annual with golden-brown inflorescence, abundant around pools, Engabao (11,032); between La Libertad and Santa Elena (11,425); east of Salinas (11,412). Virginia and Arkansas, southward in tropical America to Ecuador; and in the Old World tropics. Kuekenthal cites specimens from Chanduy (*Spruce* 6417), Guayaquil (*Andersson* 34), and Puna Island (*Eggers* 14,790).

46. C. ARISTATUS Rottb. var. *inflexus* (Muhl.) Boeckl., Linnaea 35: 500. 1868. *Cyperus inflexus* Muhl. Gram. 16. 1817. Dunes and river gravels, Salinas (11,166); Punta Ayangue (11,346); Quebrada Mogollon, Piura, Peru (H&S 11,549). This is the American representative of a widespread species in the Old World. *Spruce* 6416 is cited from Chanduy by Kuekenthal (p. 505).

47. C. COMPRESSUS L., Sp. Pl. 46. 1753; Kuekenthal, op. cit., p. 156. Sandy roadsides, Salinas (11,237). A weedy species from New York southward to Tumbez, Peru (*Raimondi*); also in the Old World. Andersson collected it at Guayaquil and on Puná Island.

48. C. CORYMBOSUS Rottb. var. *subnodosus* (Nees & Meyen) Kuekenthal in Fedde, Rep. Spec. Nov. 23: 185. 1926; Kuekenthal, op. cit., p. 82. Low ground between Ancon and Santa Elena (11,182). This variety greatly resembles *C. rotundus*, but has weak, articulate culms. Mexico to Bolivia, with center of distribution in Argentina.

49. C. ELEGANS L. Sp. Pl. ed. 2. 68: 1762; Kuekenthal, op. cit., p. 164. *C. rubiginosus* Hook. f. Trans. Linn. Soc. 20: 178. 1847; Kuekenthal, op. cit., p. 484. Fresh-water seepage in the dunes, Negritos, Piura, Peru (H&S 11,578), with heads 2 cm. in diameter. Both collections belong to var. *major* Kuekenthal (op. cit., p. 165) based on *Haught* 6, from Negritos (fig. 15).

In the Galapagos Islands the species has smaller heads which resemble those of the common West Indian plant, except that the scales are frequently deep purplish red.

50. C. ESCULENTUS L. Sp. Pl. 45. 1753; Kuekenthal, op. cit., p. 116. A widely distributed variable plant, with somewhat edible tubers. Low ground between Ancon and Santa Elena (11,181); borders of pools between La Libertad and Santa Elena (11,422). In no. 11,181 the inflorescence is close to the typical form illustrated by Kuekenthal; in no. 11,422 the spikelets are elongate (reaching 25 × 3 mm.) and so probably represent the var. *Sprucei* C. B. Clarke (Kuekenthal, op. cit., p. 121) founded on *Spruce* 6414 from Chanduy. Kuekenthal (p. 120) cites var. *macrostachyus* Boeckl. (a similar wide-ranging plant with elongate spikelets) from the Galapagos Islands (coll. *Macrae*), but these forms, I believe, merely represent the extreme of a variable species, and are without geographic significance.

51. C. FUGAX Liebmann, Vidensk. Selsk. Skr. 5, pt. 2: 196. 1851. Abundant in dune hollows, Salinas (11,167); sandy borders of salt marsh (11,314). It was collected by Spruce (6415) at Chanduy. This dwarf annual phase, only 5–15 cm. high, closely resembles *Baur* 311 from Chatham Island, Galapagos, and is smaller than *C. Gatesii* of southeastern United States, with which it has been associated by Kuekenthal (op. cit., p. 372) as *C. polystachyus* Rottb. var. *leptostachyus* Boeckl. f. 2 *fugax* (Liebm.) Kuekenthal. The widespread species, *C. polystachyus*, originally described as a perennial from the Old World, has recently been identified with *C. odoratus* L.

52. C. LAEVIGATUS L. Mant. 2: 179. 1771; Kuekenthal, op. cit., p. 321. Wet quartz sand at head of Quebrada Mogollon, Amotape Hills, Peru (H&S 11,531). Widely distributed in tropics of both hemispheres.

53. C. OXYLEPIS Nees ex Steudel, Syn. Cyp. 25. 1855; Kuekenthal, op. cit., p. 165. Abundant in wet brackish sands, Salinas (11,312); Punta Centinela (11,263). No. 11,312 is a dwarf collection with short rays, undoubtedly representing var. *punensis* Kuekenthal in Pflanzenr. IV. 20: 167. 1936. (*C. macrocarpus* Areschoug, Eugenies Resa Bot. 120. 1910; not Boeckl.). I see no advantage in maintaining such a variety. *C. oxylepis* is a widely-distributed littoral species from the West Indies to Argentina. In the herbarium it is frequently confused with *C. confertus*. None of the material which I have collected is at all glutinous (fig. 19).

54. C. PANAMENSIS (C.B. Clarke) Britton, Jour. Wash. Acad. Sci. 15: 457. 1925. *Mariscus panamensis* C.B. Clarke, Kew Bull. Add. Ser. 8: 15. 1908, and Ill. Cyp. t. 25, fig. 4–5. 1909. Grassy summit of Chanduy Hills, 1800 ft. (11,047). The species is close to the widespread *C. tenuis* Sw. but differs in having acuminate scales. My collection belongs to var. *deformis* (Clarke) Kuekenthal, op. cit., p. 498, which has longer rays (to 10 cm.) and longer spikelets (10–16 mm. long). It has been known only from Ecuador: Chanduy (*Spruce* 865), and Puná Island (*Andersson* 38, in part), except for a single collection (*Hayes* B8) from Panama.

55. ELEOCHARIS ATROPURPUREA (Retz.) Kunth, Enum. 2: 151. 1837. *Scirpus atropurpureus* Retz., Obs. 5: 14. 1789. Borders of pools east of Salinas (11,415). Of scattered distribution in both hemispheres. This species with black achenes resembles *E. caribaea*, but is smaller in all parts. *E. minima*, a dwarf species with trigonous brown achenes, was collected by Jameson (no. 369) on the "savana of Guayaquil," and by Hitchcock (no. 20,087) at the Oil Camp between Salinas and Guayaquil.

56. E. CARIBAEA (Rottb.) Blake, Rhodora 20: 24. 1918. *E. geniculata* L. Sp. Pl. 48. 1753 (in part), not of L. Sp. Pl. ed. 2. 1762. Wet quartz sand at head of Quebrada Mogollon, Amotape Hills, Peru (H&S 11,530). The most widespread species of the genus, common in the tropics of both hemispheres.

57. FIMBRISTYLIS ANNUA (All.) R. & S., Syst. 2: 95. 1817. *Scirpus annuus* Allioni, Fl. Ped. 2: 277. 1785. Margins of pools south of Engabao (11,022). Rather tall plants (reaching 5 dm.) of a widespread species.

58. HEMICARPHA MICRANTHA (Vahl) Britton, Bull. Torrey Club 15: 104. 1888. *Scirpus micranthus* Vahl, Enum. 2: 254. 1806. Abundant in moist places among dunes, Salinas (11,313, 11,165); wet quartz sand, head of Quebrada Mogollon, Amotape Hills, Peru (H&S 11,532).

This material is very close to the type examined by me in Vahl's herbarium in 1937. Vahl says that the specimen (a single small plant 6 cm. high) came from Richard, its habitat (with a question mark) being South America. The writing on the label is obscure, but looks more like "Juss." than anything else. If that is correct, the plant may well have come from the Guayaquil area where Joseph Jussieu collected in 1735. Vahl's specimen has achenes averaging 0.67 × 0.3 mm. with brown lightly tubercled surface, the scales rather closely appressed with slightly divergent tips and the entire surface semiglutinous. *H. subsquarrosa* (Muhl.) Nees has somewhat larger spikelets and achenes, the latter averaging 0.75 mm. in length. My Ecuadorean collections have small achenes consistently only 0.5 mm. long. In this respect they are like the specimen of *Isolepis Humboldtii* R.&S. (*Scirpus sesquipollicaris*) from the Orinoco River, which I examined in the Willdenow Herbarium (no. 1200), but in which the achenes are slightly curved and resemble the seeds of *Elatine*.

ARACEAE

59. PHILODENDRON aff. OLIGOSPERMUM Engler, Bot. Jahrb. 37: 132. 1905; Engler & Krause, Pflanzenf. IV. 23Db: 94, fig. 29. 1913. Trailing on ground, river bank, Manglaralto (11,506). The cordate-ovate leaves are 2.5 dm. long; the dull orange spathe, closely enveloping the spadix, is 9 cm. long. Except for the slightly wider leaf, and the spathe and spadix twice as long as described, the plant agrees very well with the description and figures of *P. oligospermum*, which came from an unknown locality in Ecuador (coll. *Sodiro*).

LEMNACEAE

60. LEMNA MINIMA Philippi, Linnaea 33: 239. 1844. Plants about 2 mm. long and 1 mm. wide, floating in pools south of Engabao (11,024). The terete seeds, longitudinally ridged and with minute cross-partitions between the ridges, are only 0.35–0.4 × 0.2 mm. in size, and the plants agree exactly with material which I collected in the Galapagos Islands (no. 87). *Lemna perpusilla* Torr., of similar size, has relatively enormous seeds, 0.75–1.0 mm. long, flattened and with a winged margin and without surface markings. *L. minima* extends from southern United States to Chile.

BROMELIACEAE

(det. L. B. Smith)

61. AECHMAEA PYRAMIDALIS Benth., Bot. Sulphur 173. 1846; Mez, Pflanzenr. IV[32]: 140. 1934. An epiphyte with light red bracts and spinose-margined leaves, Manglaralto (11,494a). This species came originally from Guayaquil, but is known also from Balao (*Eggers* 14,059) and Balsabamba (*Sodiro* 171–4).

62. GUZMANIA MONOSTACHYA (L.) Rusby ex Mez in DC. Mon. 9: 905. 1896; Mez, p. 612. 1935. *G. tricolor* R.&P., Fl. Peruv. 3: 38, t. 261. 1802; Bentham, Bot. Sulphur 174. 1846. Epiphyte, the capsules shiny black within, Manglaralto (11,494). An abundant species extending from the West Indies and Central America to Ecuador and Bolivia.

63. TILLANDSIA COMPLANATA Benth., Bot. Sulph. 173. 1846; L. B. Smith, Field Mus. Bot. 13, pt. 1: 550. 1936. Epiphyte with flowers at apex of a slender bractless stem, Manglaralto (11,496). Collected originally at Atacames, Ecuador, but widespread from the West Indies to Bolivia.

64. T. LATIFOLIA Meyen, Reise 437. 1843; L. B. Smith, l.c., p. 555. *T. divaricata* Benth. Bot. Sulph. 174. 1845. Epiphyte, with slender acuminate leaves covered by a silvery scale-like pubescence, Punta Ayangue (11,361); abundant on the higher slopes, Chanduy Hills (11,037). This is the common bromeliad in the coastal area of Ecuador, but I saw no signs of it in the very dry regions, such as the Santa Elena peninsula. It extends southward into the Mollendo region of Peru, and may perhaps occur in Colombia, as cited by Bentham.

65. T. RECURVATA L. Sp. Pl. ed. 2. 410. 1762; L. B. Smith, l.c., p. 542. Epiphyte, near summit of Cerro Prieto, 2000 ft., Piura, Peru (H&S 11,629); *Haught* 215. The slender recurved leaves are covered with silvery scales. Extends from southern United States to northern Argentina and Chile.

66. T. STRAMINEA HBK., Nov. Gen. & Sp. 1: 292. 1816; L. B. Smith, l.c., p. 561. Epiphyte, with elongate silvery leaves and fragrant flowers, white with a dark blue border, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,514). Known from Peru and Ecuador, collected originally at Olleros, Piura. It is the only bromeliad which I saw in full flower on the Ecuadorean-Peruvian coast.

COMMELINACEAE

67. COMMELINA ELEGANS HBK., Nov. Gen. & Sp. 1: 259. 1816.

A tuberous-rooted, blue-flowered perennial in thickets along streambed about 5 miles west of Chanduy Hills (11,001). The capsule is 2-celled, each cell with a single seed 3 × 2.5 mm. One cell of the capsule is smooth and dehiscent, the other indehiscent with a conch-like echinate surface. The collection is close to *Killip & Smith* 14,066 from Cartagena, Colombia; and the species was reported (as *C. virginica*) from Guayaquil by Bentham. *Commelina elegans* belongs to the section *Hetero-*

pyxis Clarke, which has the peculiar type of capsule described above, and no. 11,001 appears to be identical as to capsule and seeds with *C. crispa* Wooton of southwestern United States. But as Pennell (Bull. Torrey Club 43: 105. 1916) has stated in treating *Commelina erecta* L.: "Capsules with posterior cell more or less reduced and tardily dehiscent, a state which has been separated as 'Commelina virginica,' are to be found in the same colonies and even upon the same plants with capsules normally developed, three-celled and evenly dehiscent."

PONTEDERIACEAE

68. HETERANTHERA LIMOSA Vahl, Enum. 2: 44. 1806; Solms-Laubach in DC. Mon. 4: 518. 1883. Cordate-leaved blue-flowered annuals floating on pools, south of Engabao (11,030). The species extends from central United States to Paraguay and Argentina. It was collected by Spruce at Chanduy.

LILIACEAE

69. SMILAX TUBERCULATA Presl, Rel. Haenk. 1: 132. 1825. Stout vine climbing over bushes at Manglaralto (11,449, det. Killip). This little-known species has been collected previously only at Guayaquil.

AMARYLLIDACEAE

70. HYMENOCALLIS QUITOENSIS Herbert, Amaryll. 218, t. 22, f. 4. 1837. Locally between Santa Elena and Ancon (11,105a); open woods, ridge north of Santa Elena (11,105, in fruit). Well represented by Herbert's figure which was based on a Pavon specimen in Lambert's herbarium. It is well known (cf. Diels, p. 54) that Pavon's Ecuadorean specimens came mostly from his student, Tafalla, who collected chiefly in the Guayaquil region. Baker, Handbook Amaryll. 127, 1888, says that Pavon's specimen came from the "Cerro of Santana, Guayaquil," now within the modern city limits.

With this plant Baker confused a wholly distinct species which he had published in the Botanical Magazine, t. 6397. 1878, as *Ismene tenuifolia,* and which has short filaments, small anthers, and a cleft and dentate staminal tube—a plant certainly close to, if not identical with, the Peruvian *Hymenocallis pedunculata* (Herbert) Macbride, Field Mus. Bot. 11: 11. 1931. In *H. quitoensis* the tube is entire, the filaments project approximately 3 cm. beyond its margin, and the anthers are 10–12 mm. long. Thus it is representative of the genus *Hymenocallis* and not at all of *Pamianthe* (under which Stapf in Bot. Mag. sub. t. 9315. 1933, apparently innocent of Baker's mistake, has it as *P. quitoense*). The obscurely-trigonous soft green-striate capsules have translucent brown seeds which frequently germinate within the capsule. *H. quitoensis* is widespread in the richer lowlands of southwestern Ecuador, and extends to the Peruvian coast in the Tumbez region (cf. Field Mus. Bot. 13, pt. 1: 673. 1936. coll. *Weberbauer*). Early in February the savannas east of Santa Elena are white with countless thousands of the large fragrant flowers. By the end of the month all have disappeared, and in March I was unable to find even a trace of the leaves.

ORCHIDACEAE (det. C. Schweinfurth)

(No orchids were observed in flower in this region.)

71. CAMPYLOCENTRUM aff. ECUADORENSE Schltr. Occasional epiphyte, Chanduy Hills, 1600 ft. (11,055).

72. EPIDENDRUM *Sect. Encyclium.* Abundant at summit, Chanduy Hills, 1800 ft. (11,048).

73. ONCIDIUM sp. Chanduy Hills, 1600 ft. (11,013).

PIPERACEAE

74. PEPEROMIA aff. PENDULICAULIS C.DC., Ann. Conserv. Jard. Bot. Genève 21: 266. 1920. Epiphytic, with alternate broadly lanceolate leaves and filiform inflorescence, Manglaralto (11,458). *P. pendulicaulis* was described from Balao, *Eggers* 14,680 (of which I have seen only a photograph). The leaves in no. 11,458 are considerably larger, the blades reaching 22 × 10 mm., the petioles (up to 5 mm.) are much longer, and the inflorescence reaches 5 cm. in length. This plant differs from *P. petiolata* Hook. f. of the Galapagos Islands in its acuminate leaves, shorter and pubescent petioles, and thinner spikes. Except for shorter spikes, no. 11,458 closely resembles the Brazilian *P. myrtifolia* f. *latifolia* Miguel, Linnaea 20: 122, t. 1, f. 2. 1847.

75. P. aff. ROTUNDIFOLIA (L.) Dahlstedt, Vet. Akad. Handl. 33, pt. 2: 99, t. 11. 1800; Macbride, Field Mus. Bot. 13, pt. 2: 87. 1936. *Piper rotundifolium* L. Sp. Pl. 30. 1753. Epiphyte, with long pendent branches and rounded leaves 1 cm. wide, Chanduy Hills, 1600 ft. (11,026 & 11,036). In plants where the pendent branches are undeveloped, rhombic leaves occur which are as large as 2.5 × 2.0 cm. This is the only *Peperomia* which I saw in the dry area of Ecuador, and I could find no flowers. *P. rotundifolia* is of wide distribution from the West Indies to South America.

76. PIPER ADUNCUM L. Sp. Pl. 29. 1753; Fawcett & Rendle, Fl. Jamaica 3, pt. 1: 24. 1914; Britton & Wilson, Surv. Porto Rico & Virgin Isl. 5: 221. 1924. Trees 20 ft. tall, with pinnate-veined acuminate leaves, and recurved spikes. Stream banks, Manglaralto (11,445). Widespread from Cuba to tropical South America.

77. P. MEDIUM Jacq., Ic. Rar. 1: 2, t. 8. 1781; DC., Prod. 16, pt. 1: 246. 1869; C.DC., Candollea, 1: 76. 1923. Shrubs 6–8 ft. high, with palmate-veined leaves. Stream banks, Manglaralto (11,492). Mexico and the West Indies to Brazil.

78. P. TUBERCULATUM Jacq., Collect. 5: 7 (1786–1796); Fawcett & Rendle, Fl. Jamaica 3, pt. 1: 22. 1914. Shrubs to small trees, the young branches rough, and the leaf-bases very unequal, Manglaralto (11,476, det. Killip, & 11,491). Cuba to Colombia and Brazil.

None of these three species of *Piper* are included by Trelease in Macbride, Fl. Peru 2: 107–253. 1936.

URTICACEAE

79. Myriocarpa stipitata Benth., Bot. Sulphur 168, t. 55. 1846; Macbride, Field Mus. Bot. 13, pt. 2: 360. 1937. A shrub 5 ft. high in dense forest, Manglaralto (11,507). This species was described from the coast at Tumaco, Colombia, just north of the Ecuadorean boundary. It extends from Venezuela to southern Peru, where it is a plant of the Amazon watershed.

80. Parietaria debilis Forst., Fl. Ins. Austr. Prod. 73. 1786. Macbride, Field Mus. Bot. 13, pt. 2: 366. 1937. Shaded rocks near summit of Cerro Prieto, Piura, Peru (H&S 11,633). The seeds of this sprawling weed are only 0.7 × 0.4 mm., somewhat smaller than generally found in plants of the Old World tropics.

81. Pilea microphylla (L.) Liebm., Dansk. Vid. Selsk. Skrivt. V. 2: 296. 1851; Killip, Contrib. U. S. Nat. Herb. 26: 477. 1939. An epiphyte with somewhat fleshy leaves, Manglaralto (11,439). This widely distributed species, in the American tropics, is cited from Guayaquil (*Mille*) by Killip.

LORANTHACEAE

82. Psittacanthus linearis (Killip) Macbride, Field Mus. Bot. 13, pt. 2: 406. 1937. *Phrygilanthus linearis* Killip, Jour. Wash. Acad. Sci. 24: 45. 1934. Parasite on *Prosopis,* Quebrada Ancha, near La Brea, Peru (H&S 11,574). This plant grows with *P. obovatus* and has exactly the same type of branches and flowers, but the leaves are linear and the pedicels lack the minute puberulence associated with *P. obovatus.* I suspect that *P. linearis* is a narrow-leaved variant of *P. obovatus.*

83. P. obovatus Benth., ex Eichl. in Mart. Fl. Bras. 5, pt. 2: 27. 1868; Macbride, Field Mus. Bot. 13, pt. 2: 407. 1937. *Loranthus obovatus* Benth. Bot. Sulphur 103. 1844. Orange-flowered parasite on leguminous trees, low ground north of Ancon (11,429); near summit, Chanduy Hills, 1600 ft. (11,010); Quebrada Ancha, near La Brea, Peru (H&S 11,587). This species was also collected by Haught (no. F 22) at Negritos. Originally known from Guayaquil, the leaves were described as "subsessilibus obovatis obtusis basi rotundatis cuneatisve venosis." In my collections the leaves are practically sessile and vary from elliptic to obovate. There is little doubt that *P. chanduyensis* Eichler, in Mart. Fl. Bras. 5, pt. 2: 27. 1868, with sessile leaves, is the same as *P. obovatus.*

POLYGONACEAE

84. Coccoloba Ruiziana Lindau, Bot. Jahrb. 13: 215. 1890; Macbride, Field Mus. Bot. 13, pt. 2: 461. 1937. A small tree, to 15 ft. high, with irregular horizontal branches in low ground between Ancon and Santa Elena (11,179); between Santa Elena and Punta Centinela (11,276). This species was collected by Spruce at Chanduy (no. 6340) and by Andersson on Puná Island (cf. Lindau). (Areschoug, Eugenies Resa Bot. III: 124. 1910, says that Andersson's specimens conform with material of *C. marginata* Jacq. in the Copenhagen herbarium). It is also known from Peru from the original collection of Ruiz & Pavon (locality unknown) and from Departments of Lambayeque, Piura, and Tumbez (*Weberbauer* 7738).

CHENOPODIACEAE

85. Chenopodium murale L. Sp. Pl. 219. 1753; Macbride, Field Mus. Bot. 13, pt. 2: 474. 1937. A weed with triangular leaves in waste places by the seashore, Salinas (11,068). The nearly black seeds are 1.3 mm. wide, with surface minutely spinescent-papillose. An Old World plant widely spread in both North and South America.

AMARANTACEAE

Alternanthera Forsk., Fl. Aegypt. 28. 1775; Howell, Proc. Calif. Acad. Sci. ser. 4, 21: 99–113. 1933. This genus is one of the most complicated on the Galapagos Islands, but many of the supposed endemic species are quite obviously on the mainland. A number of these plants are shown in the accompanying photographs (Pls. 8, 9 & 10). It is an exceedingly difficult group for study, especially the section *Bucholzia,* since the flowers mature irregularly. Relatively few can be found suitable for dissection, for either the staminodia have not yet elongated or they have become withered. Mature seeds, which might be important from the point of view of specific differentiation, are very few in collections. The flowers are borne compactly in ovate to elongate heads. Each flower consists of five indurated, pointed sepals of varying size forming a somewhat elongate perianth; the two innermost are usually keeled and inclose the stamens and staminodia. Three bracts, frequently spiny-pointed, and about half as long as the sepals enclose the flower.

By Schinz in Engler, Pflanzenf. 16c: 71. 1934, the genus is divided into the following groups:

Subgen. *Eualternanthera* Graebner. Filaments elongate. Pseudostaminodia small and ligulate, rarely dentate.

Section 1. *Allaganthera* (Mart.) Moq. Inflorescence axillary. Stamens 3–5. Procumbent plants, including *A. paronychioides, A. repens,* and *A. pungens.*

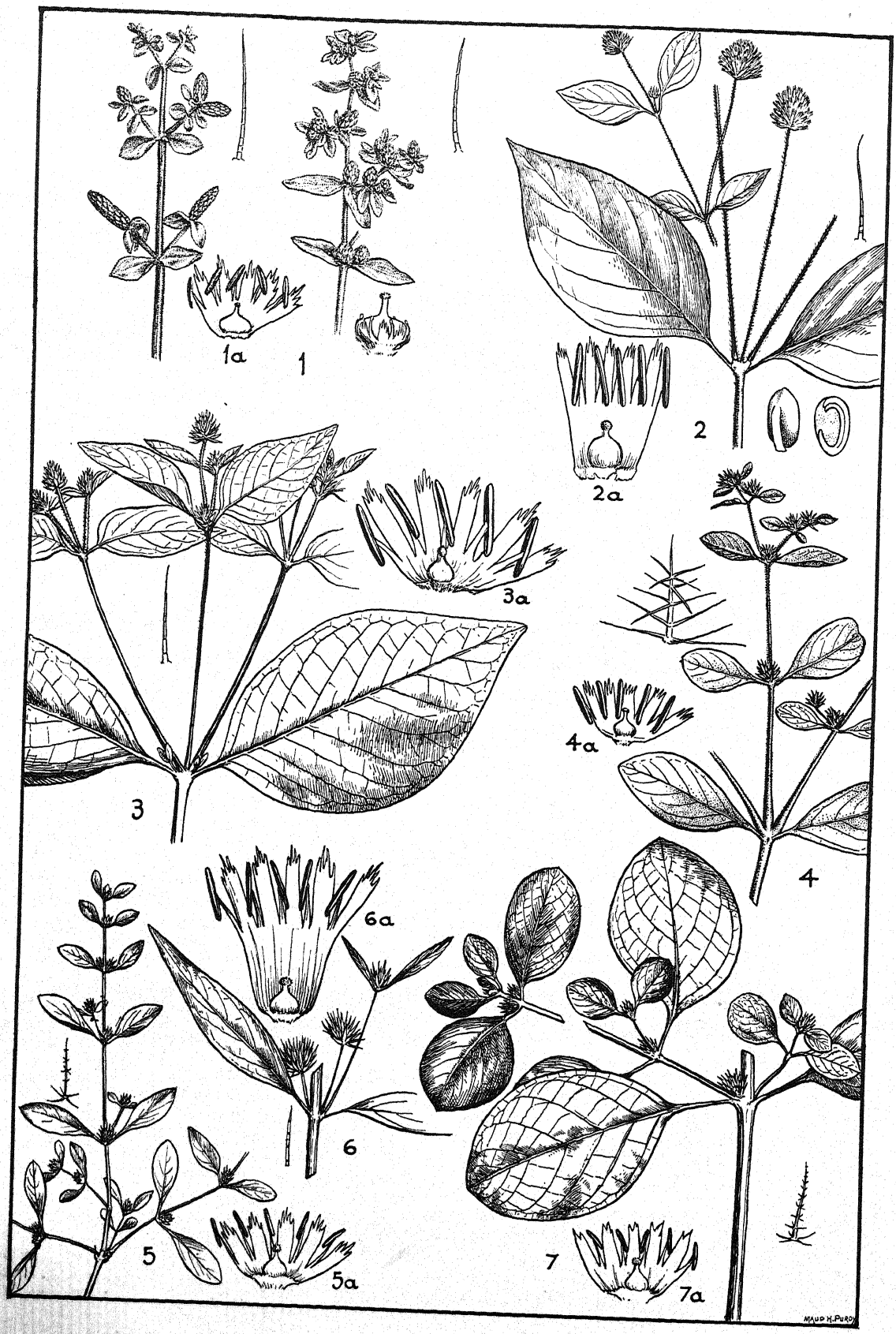
Section 2. *Dassiera* Moq. Inflorescence terminal. Stamens 2–3. Erect plants, including *A. subscaposa* of the Galapagos Islands, which Howell (op. cit., p. 97) has treated better as *Lithophila subscaposa.*

Subgen. *Telanthera* (R.Br.) Graebner. Staminal tube often elongate, the pseudostaminodia fringed.

Section 1. *Bucholzia* (Mart.) Schinz. Inflorescence axillary and terminal. Calyx not stipitate. Includes *A. ficoidea, A. halimifolia,* and *A. philoxeroides.*

Section 2. *Brandesia* (Mart.) Schinz. Calyx stipitate, the stipe articulate at the base. Includes *A. pubiflora* and *A. echinocephala.*

Section 3. *Mogiphanes* (Mart.) R.E. Fries. Heads long-pedunculate. Calyx with a columnar base, the bracts often dentate. Includes *A. dentata* and *A. brasiliana* (Pl. 6, fig. 2; Pl. 7, fig. 4b).


1a
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2a
3a
3
4a
4
6a
6
5
5a
7
7a
MAUD H. PURD

86. A. ECHINOCEPHALA (Hook. f.) Christophersen, Nyt. Mag. Naturvid. 70: 73. 1932; Howell, Proc. Calif. Acad. Sci., ser. 4, 21: 110. 1933. *Brandesia echinocephala* Hook. f., Trans. Linn. Soc. 20: 189. 1847. *A. tubulosa* Suessenguth, Rep. Spec. Nov. 42: 56. 1937; Standley, Field Mus. Bot. 13, pt. 2: 1135. 1938.

A. echinocephala, one of the most abundant species of the Galapagos Islands, is identical with *A. tubulosa,* described by Suessenguth from the Peruvian coast from Lima to Pisco, but also extending to the vicinity of Arequipa (fig. 17). I have seen the following specimens (NY): south of Santa Clara, Peru, *Rose* 18,622, July 6, 1914; vic. Chosica (850 m. alt., near Lima, see Rose, Jour. N.Y. Bot. Gard. 16: 171. 1915), Peru, *Rose* 18,544, June 30, 1914; Pisco, Peru, *Rose* 11,803, Aug. 6, 1914; near Tingo, vic. Arequipa, Peru, *Rose* 18,800, Aug. 5, 1914; interior of Peru, *Matthews* in 1842 (cf. Pl. 6, fig. 6; Pl. 9, fig. 1, 2).

87. A. FICOIDEA (L.) R.&S. Syst. 5: 555. 1819; Fawcett & Rendle, Fl. Jam. 3, pt. 1: 140. 1914. *Gomphrena ficoidea* L. Sp. Pl. 225. 1753. *Illecebrum ficoideum* L. Sp. Pl. ed. 2, 300. 1762. *A. polygonoides* Griseb., Fl. Br. W. Ind. 67. 1859.

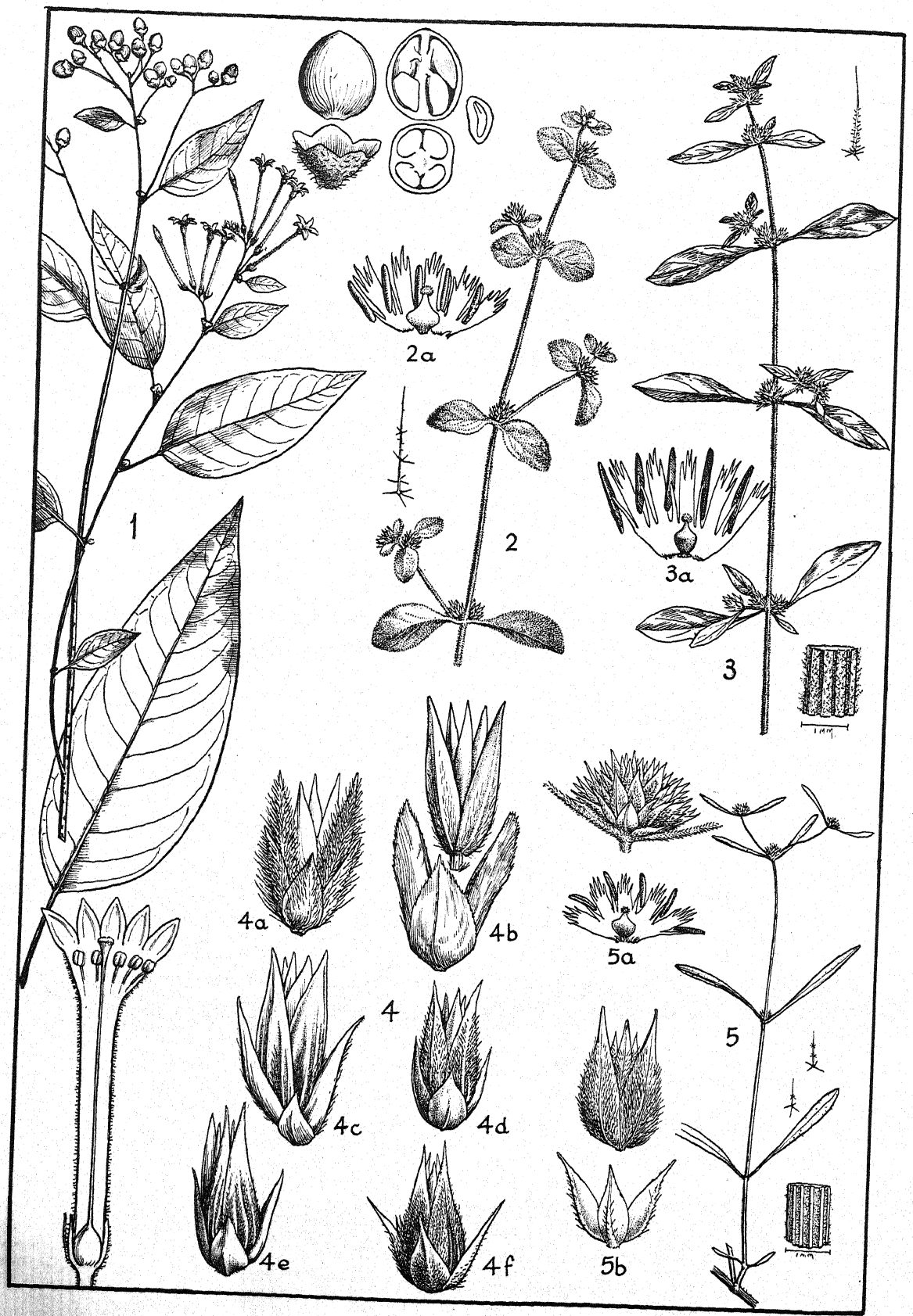
A. ficoidea has undoubtedly been the source of more confusion than any other species of *Alternanthera,* due to its close resemblance to *A. polygonoides.* Linnaeus more clearly distinguished the two species in the second edition of Species Plantarum, *Illecebrum ficoideum* being treated as a plant with broadly-ovate leaves and obviously pubescent acute corollas, while in both species the heads were said to be rounded. *I. polygonoides* was distinguished by having villous stems and *smooth* flowers. Here, Burmann's edition of a Plumier plate was included under *I. polygonoides* as β *Amaranthoides marina hirsuta, halimi folia,* a name which later received formal status as *Telanthera polygonoides* ˢ *major* Moquin in DC., Prod. 13, pt. 2: 364. 1849. For the ensuing confusion, I suspect Lamarck is to blame. He gave a new name, *Achyranthes halimifolia,* to "Illecebrum limense Hort. Reg.," based on Dombey's Peruvian plants then cultivated at Paris. To this, as var. β, was appended the Linnaean citation of *Amaranthoides marina hirsuta, halimi folia* and it was said to be a plant of sandy maritime shores of the Antilles, differing from the Peruvian plant in having stems more abundantly pubescent and with only two leaves to a node because of the undeveloped axillary shoots. *Alternanthera ficoidea* is widely distributed throughout the West Indies as a decumbent plant with thin lanceolate-ovate acute leaves, usually 2–3 cm. wide. The leaves may rarely become as narrow as 1 cm. as in some Grisebach citations from Jamaica. This narrow phase is the only one which I have seen from the Galapagos Islands, where it is clearly represented by such collections as *Stewart* 1386 from 900–1400 ft. on Abingdon Island [det. *"A. flavicoma"* by Stewart; det. "A broad-leaved form of *A. flavicoma* ?" by Howell] and by *Stewart* 1396 from 2100 ft. on James Island [det. *"A. flavicoma"* by Stewart; det. *"A. halimifolia macrophylla,* form with leaves narrower than usual" by Howell] (Pl. 8, fig. 1, 2; Pl. 7, fig. 3, 4f).

The leaves of *A. ficoidea* were described by Linnaeus as "lanceolato-ovatis" and "lato lanceolatis." By Fawcett & Rendle, the leaves are said to be 3–7 cm. long and 1–3 cm. broad and glabrate, the tomentum of the young leaves sometimes partially remaining. On seacoasts of the West Indies and northern Colombia and Venezuela, *A. ficoidea* runs into a form (var. *flavogrisea*) with coriaceous leaves varying from acute to rounded at the apex. These frequently have the livid appearance associated with *Heliotropium curassavicum,* and both surfaces are often covered with a dense appressed yellow-to-white pubescence. Frequently the heads are elongate. It is well shown by the extensive series collected by *Curran & Haman* in Venezuela at Vela de Coro, opposite the island of Curaçao (nos. 441, 446, 452, 452A, 456), in the Gray Herbarium; also by *Maxon & Killip* (nos. 339 and 1393) from Kingston, Jamaica; and by a collection from Chemperico, Yucatan (*Standley* 66,515). There is a tendency for this plant to run inland, as in the Lititz Savanna, Jamaica, alt. 300–900 ft. (*Harris* 11,761 & 11,762); and in *Pittier* 8632 from Carabobo, Venezuela (20–100 m. alt.). Fawcett & Rendle seem to be perfectly correct in treating this plant as a variety of *A. ficoidea,* and its synonymy is as follows:

A. ficoidea var. *flavogrisea* Fawcett & Rendle, Fl. Jamaica 3: 140. 1914. *Telanthera flavogrisea* Urban, Symb. Ant. 1: 300. 1899. *Alternanthera canescens* HBK., Nov. Gen. & Sp. 2: 204. 1818. *?Illecebrum Humboldtianum* R.&S., Syst. 5: 517. 1819. *?Telanthera Crucis* Moq. in DC., Prod. 13, pt. 2: 362. 1849. *Achyranthes halimifolia* sensu Standley, N. Am. Fl. 21: 139. 1917, as to Caribbean plants only.

Judging from Moquin's treatment, *A. Crucis* is based primarily on West's collection from St. Croix. However, Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 5: 281. 1924, state that *Achyranthes halimifolia*—as Standley treats the plant—is unknown in the Virgin Islands, but is abundant on Curaçao. *A. canescens* (from Cumaná, Venezuela) is a narrow-leaved extreme of var. *flavogrisea,* in which the revolute linear leaves are usually only 1–2 mm. wide and do not exceed 1 cm. in length.

Plate 6. *Alternanthera* from coast of Ecuador and Peru, and from Galapagos Islands. The staminal tube (a) × 4 is opened to show relative length of tube, anthers, and pseudostaminodia. Simple and stellate hairs are enlarged.—Fig. 1. *A. peruviana* (*Haught & Svenson* 11,603, Peru) showing rounded and elongate spikelets; perfect flower (1a) from *Pennell* 12,203.—Fig. 2. *A. dentata* (11,018, Ecuador).—Fig. 3. *A. pubiflora* (11,580, Ecuador).—Fig. 4. *A. truxillensis* (*H&S* 11,622, Peru).—Fig. 5. *A. halimifolia* subsp. *macrophylla* (*Svenson* no. 229, Galapagos I.).—Fig. 6. *A. echinocephala* (*Svenson* no. 4, Galapagos I.).—Fig. 7. *A. halimifolia* subsp. *macrophylla* (*Svenson* no. 103, Galapagos I.).


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1 MM
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4b
4
4c
4d
4e
4f
5a
5
5b
1 MM

It is well represented by *G. P. Cooper* 212 (NY) from Cabagua Island, and is cited by J. R. Johnston (Proc. Boston Soc. Nat. Hist. 34: 206. 1909) from the nearby islands of Margarita and Coche, Venezuela. *Curran & Haman* 441 (one of the series already cited from Vela de Coro, Venezuela) is clearly transitional from var. *flavogrisea* to *A. canescens*.

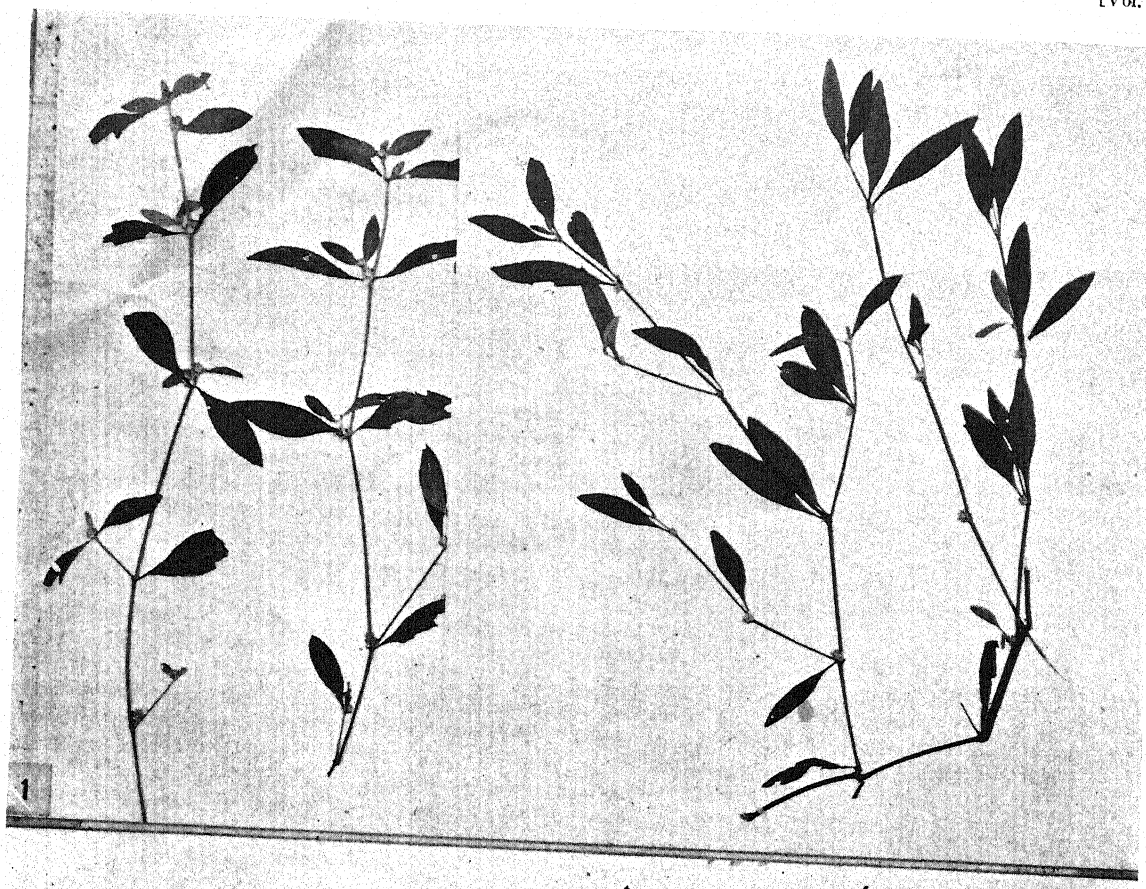
The Galapagos collection which I called *A. ficoidea* (*Svenson* 229), I now refer to *A. halimifolia* subsp. *macrophylla*. To return to the Stewart collections: no. 1368, except for a little less pubescence, is identical with *March* 878 from Jamaica (cf. Pl. 8, fig. 1). It passes into the more pubescent no. 1415 (originally distributed as *T. Snodgrassii*) and no. 1392 from 550 ft. alt. at Villamil, Albemarle Island. All these I place under typical *A. ficoidea,* in which the leaves vary from pale bluish-gray to green, and the outer sepals are acuminate, firm and sharp-pointed, pubescent to nearly glabrous; inner sepals are strongly 3-ribbed, with yellowish pubescence which has short side branches; anthers are about 1.3 mm. long (when moist) and the staminodia (equalling the anthers in length) are 2.5 mm. long and 0.5 mm. wide, with 6–10 deeply-incised teeth at the apex.

A. halimifolia is closely related to *A. ficoidea* but appears to be a distinct species confined to the west coast of South America. The leaves are always rounded at the apex—sometimes, as in var. *nummularia,* becoming orbicular. The anthers are usually smaller (about 1.0 mm. long), and the staminodia a little shorter (2.0 mm. long) and narrower than in *A. ficoidea,* with 4–6 less deeply cut, apical teeth. I have no difficulty in separating out the two species from their general appearance.

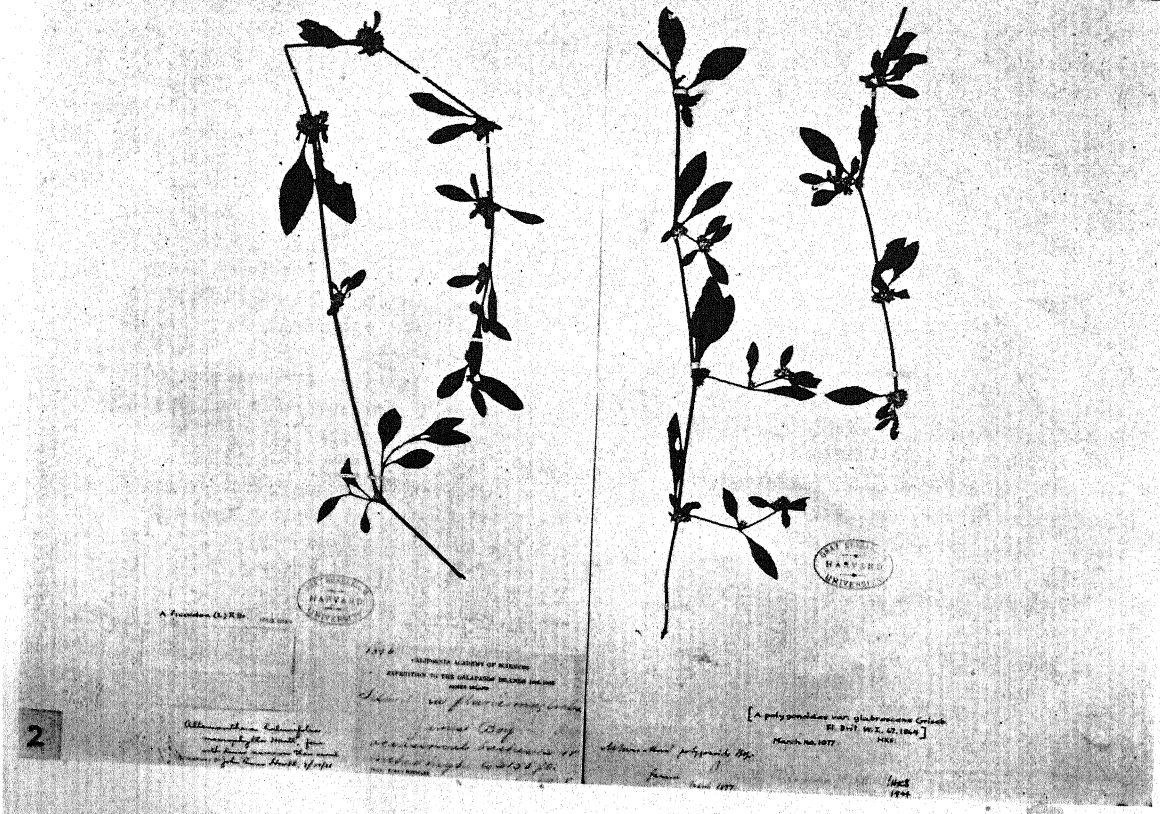
Exactly the same flower structure as in *A. ficoidea,* but on a slightly smaller scale is seen in specimens of *A. filifolia* (such as *Howell* 9443 from Albemarle Island). Here the anthers are 0.5 mm. long and the staminodia 1.5 mm. long. My collection no. 16 from Indefatigable Island has been treated as a pubescent variant of *A. filifolia sylvatica* by Howell (p. 104) and he has noted that it is "an ambiguous plant perhaps nearest to *A. flavicoma,* but with leaves a little too broad." The leaves of this plant reach 6 mm. in width, and the inflorescence is exactly that of *A. ficoidea* as represented by Broadway's collection (June 11, 1920) from Trinidad (G). I am of the opinion that it is transitional between *A. ficoidea* as already cited by me from the Galapagos Islands (*Stewart* 1396 etc.) and *A. flavicoma* as represented by *Stewart* 1392 from Albemarle Island and *Stewart* 1397 from James Island. Furthermore I think that these are transitional to the very narrow-leaved *A. nudicaulis* and *A. filifolia,* and we may have a series of reduced leaf variations derived from *A. ficoidea,* similar to the situation in *Croton Scouleri, Waltheria ovata,* and numerous other species in the Galapagos Islands. If we could know the exact ecological conditions under which these variants grow, I think we would go far toward the solution of the species problem (as regards plants) in the Islands. In discussing *A. flavicoma,* Howell (p. 107) mentions a collection in the Gray Herbarium labeled "S. Chili, *J.G. Reynolds,*" which he thinks may be from the Galapagos. I am of the same opinion. There are two other sheets of this collection in the Gray Herbarium; one with the annotation, "Andes, South America," the other, "Andes, *Capt. Reynolds*" (Pl. 7, fig. 3, 5).

88. A. HALIMIFOLIA (Lam.) Standley ex Pittier, Pl. Usual. Venez. 145. 1926, and in Macbride, Fl. Peru, pt. 2: 499. 1937. *Achyranthes halimifolia* Lam. Encyc. 1: 547. 1785, and Ill. t. 168, fig. 2. 1797. *Illecebrum limense* Lam. (l.c.) (nomen in synonymy); DuMont de Courset, Bot. Cult. 1: 646. 1802. *?Illecebrum frutescens* L'Hér. Stirp. Nov. 75. 1788. *Alternanthera albotomentosa* Suessenguth, Rep. Spec. Nov. 42: 54. 1937; Standley, Fl. Peru, 13, pt. 2: 1134. 1938. Partly submerged at margin of a dune-pond at the Puntilla, Salinas (11,158 & 11,073); in moist cultivated ground, Salinas (11,283); edge of a salt marsh, Punta Ayangue (11,349). In some specimens from the Puntilla the leaves are all 1 cm. or less in length. These collections are the same as *Hitchcock* 20,106 from the sandy bank of a stream between Salinas and Guayaquil, and the equivalent of other collections from the coast of Peru and Chile. *A. halimifolia* was grown at Paris from Dombey's collection near Lima. It was described by Lamarck as a creeping plant with greenish-white leaves covered, especially when young, by short, almost farinaceous pubescence. It was also noted as closely resembling *Glinus dictamnoides* L. [Mollugo hirta], but with leaves more elongate. *Illecebrum frutescens* L'Her. Stirp. Nov. 75. 1788, is generally included, but from the illustration it would appear to be a much coarser shrubby plant with larger leaves, and is perhaps *A. pubiflora. A. halimifolia* is well represented by the following specimens [Gray Herbarium]: Chile: Prov. Atacama, Castilla, alt. 300 ft., *Werdermann* 467; Prov. Tacna, Azapa, *Werdermann* 714; Arica, *Jaffuel* 3905; *Shepard* 274. Peru: Salaverry, *I.M. Johnston* 3521; Lima, *Grant* 7409; San Lorenzo I., Callao, *Andersson* in 1852; and the "Ecua-

Plate 7.—Fig. 1. *Cestrum auriculatum* (11,453, Ecuador), with flower in longitudinal section × 2.5; details of calyx and fruit above.—Fig. 2. *Alternanthera halimifolia* (*Werdermann* 714, Chile); dissected flower (2a) × 4.—Fig. 3. *Alternanthera ficoidea* (*Wilson* 498, Jamaica); dissected flower (3a) × 4. This is a narrow-leaved form labeled by Grisebach *A. polygonoides* var. *a.*—Fig. 4. Single flowers of *Alternanthera* × 5; perianth of 5 unequal sepals with 3 bracts at base: a, *A. pubiflora* (11,186, Ecuador); b, *A. dentata* (11,018, Ecuador); c, *A. halimifolia* (11,418, Ecuador); d, *A. halimifolia* (*Werdermann* 467, Chile); e, *A. truxillensis* (*H&S* 11,594, Peru); f, *A. ficoidea* (*March* 878, Jamaica).—Fig. 5. *Alternanthera flavicoma sylvatica* (*Svenson* 16, Galapagos Islands); flowering branch × 2; dissected flower (5a) × 2; sepals and 3 bracts (5b) × 5.



1



2

dorean" collection by Seemann. The round-leaved *Telanthera frutescens* var. *nummularia* from "hort. genev." is approached by Ball's collection from 1500–6000 ft. in the Rimac Valley, Peru.

As in most species of *Alternanthera,* the amount of pubescence on the sepals varies. The sepals are almost invariably more white-tipped than in the West Indian *A. ficoidea,* and the hairs themselves are whitened, not yellowish. *A. halimifolia* extends from Chile northward along the coast. I have seen no specimens north of Ecuador, and none (except subsp. *macrophylla*) from the Galapagos Islands. *A. albo-tomentosa* based on *Pennell* 14,456 from Rio Chillon, alt. 1800–2000 m., Dept. Lima, is unusually white-pubescent; but otherwise not different from *Eyerdam* 9125 (from near sea level at Prov. Chancay, Dept. Lima), which is a good representative specimen of *A. halimifolia* (Pl. 7, fig. 2, 4 c, d).

A. Helleri (Rob.) Howell, Proc. Calif. Acad. Sci., ser. 4, 21: 109. 1933, supposed to be endemic to Culpepper and Wenman Islands in the Galapagos, is found also at Arica on the coast of Chile (*Jaffuel* 1620 (G)), (Pl. 10, fig. 1). In all details this appears identical with *Stewart* 1414 from Culpepper Island. As a species it is close to *A. halimifolia.*

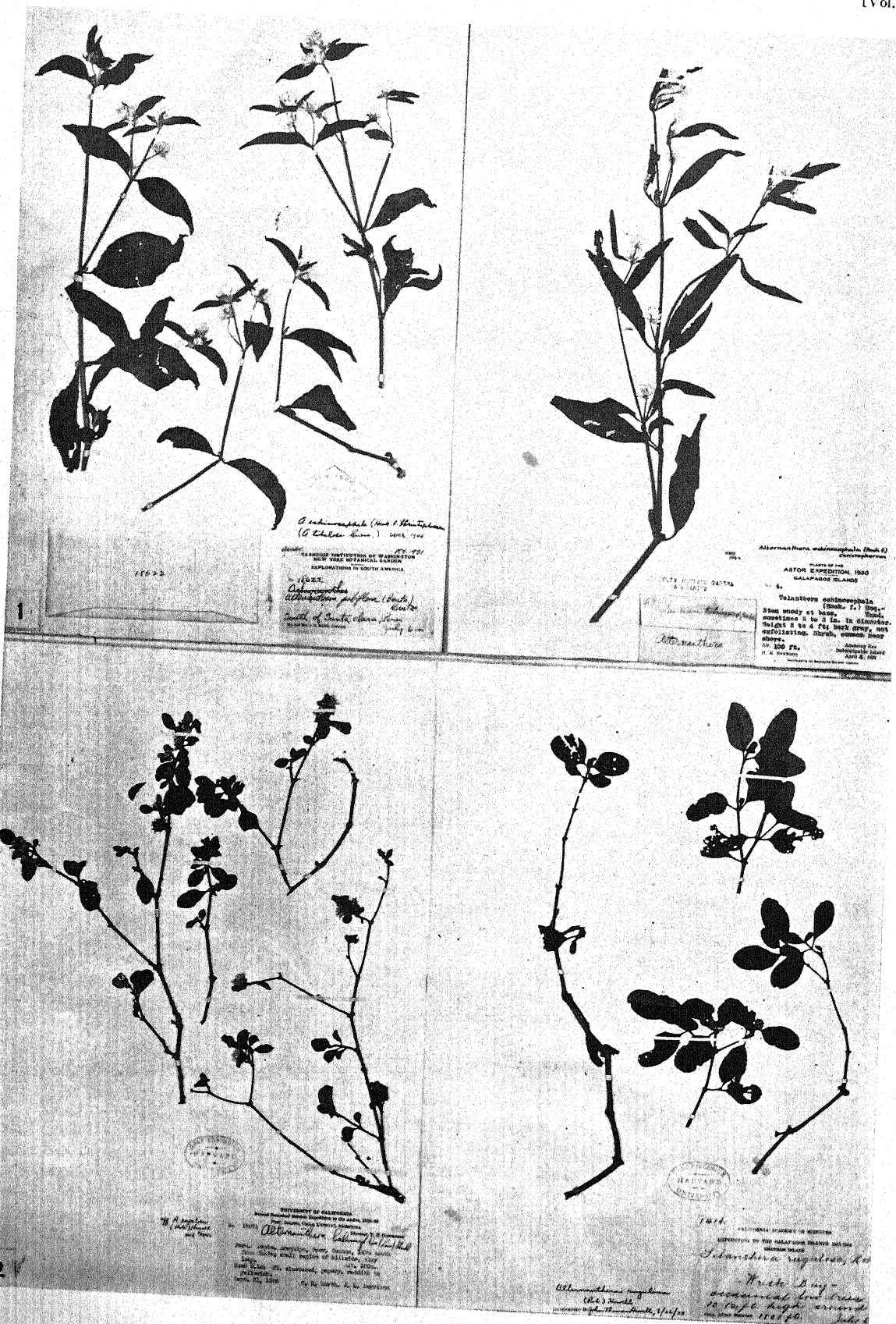
89. A. HALIMIFOLIA subsp. MACROPHYLLA Howell, Proc. Calif. Acad. Sci., ser. 4, 21: 112. 1933. *A. ficoidea* sensu Svenson, Am. Jour. Bot. 22: 229. 1935; not (L.) R.Br. Prostrate to ascending plants on ridge north of Santa Elena (11,374); open woods, Punta Ayangue (11,351); low woods, north of Ancon (11,418); Serran, alt. 200–300 m., Piura, Peru, *Weberbauer* 5977 (G). This is the characteristic species of *Alternanthera* encountered in the more mesophytic woodlands of the Galapagos Islands and reaches its greatest development in the moist region of Indefatigable Island (cf. Pl. 6, fig. 5, 7). Specimens collected at Santa Elena (no. 11,374) at an early time in the growing season (Feb. 22) had blades as much as 7 × 4 cm. and it is probable that the plants increased in luxuriance through the month of March. From the growth of *Alternanthera, Croton,* and other plants characteristic of the Galapagos Islands one might infer that the northern slopes of the ridge at Santa Elena are the equivalent climatically of a 400 ft. elevation at Academy Bay. *A. halimifolia* subsp. *macrophylla* is a sprawling woodland plant. I did not find it at the borders of salt marshes and fresh to brackish ponds, the habitat of *A. halimifolia* in western Ecuador. It is characterized especially by the rugose pubescent stem, long internodes, sparse inflorescence which is present to some degree in nearly all the leaf axils, and a peculiar grayish-yellow velvety pubescence on the young growth, whereas the older leaves become nearly glabrous. At its lower limit of growth in the Galapagos Islands the plant may become much reduced in size, the leaf shape simulating that of *A. ficoidea* (cf. Pl. 6, fig. 5). In well-developed inflorescences (as in no. 11,351) the perianth is whitened, about 5 mm. long, and nearly glabrous. It ranges from northwest Peru and western Ecuador to the Galapagos Islands.

90. A. PERUVIANA (Moq.) Suessenguth, Rep. Spec. Nov. 35: 302. 1934; Standley, Field Mus. Bot. 13, pt. 2: 504. 1937. *Telanthera peruviana* Moq. in DC., Prod. 13, pt. 2: 366. 1849. Coastal dunes, Negritos, Piura, Peru (H&S 11,603). This sprawling species is characterized by a silvery pubescence, occasionally yellowish. The heads range from globular (f. *globifera* Suessenguth l.c., based on Ball's collection from Paita) to long-cylindric, reaching a length of nearly 2 cm. (cf. Pl. 6, fig. 1). Suessenguth was unable to find any anthers in the specimens which he investigated, nor do any appear in no. 11,603. Only poorly-developed filaments and staminodia are present and usually the utricles fail to develop. Only a few were in mature condition, one of which is shown together with the rigid perianth-segments in Pl. 6, fig. 1. In *Alternanthera,* in general, the small number of adequately developed flowers makes a study of the genus difficult. Suessenguth (l.c.) was unable to find any stamens, and considers *A. peruviana* as perhaps dioecious; but they were included in the original description, and I have found complete flowers in *Pennell* 12,203, of which one is figured in Pl. 6, fig. 1a. *A. peruviana* seems to be confined to the northwest coast of Peru. At the Gray Herbarium I have seen *Haught* 32, *Johnston* 3508, and *Pennell* 12,203 (issued as *A. ochracea*) from Talara; and *Weberbauer* 7749, from between Zorritos and Cancas, Tumbez. A *Seemann* collection (G) is cited from Ecuador by Suessenguth. This specimen has no further location on the label and it probably came from Paita. Seemann replaced Edmonstone, who perished near Atacames on the Ecuadorean coast, while botanist of the "Herald." Seemann met the "Herald" at the city of Panama upon his return from Vancouver, and later went "from Payta through the Peruvian deserts, and across the Cordillera of the Andes to Loja, Cuenca, and Guayaquil" (cf. Jour. Bot. 10: 2. 1872).

91. A. PUBIFLORA (Benth.) Kuntze, Rev. Gen. 538. 1891. *Brandesia pubiflora* Benth., Bot. Sulphur 157. 1844 and var. *glomerata* (l.c.). *Achyranthes Williamsii* Standley, Contrib. U.S. Nat. Herb. 18: 89. 1916; Suessenguth, Rep. Spec. Nov. 42: 57. 1937. *Alternanthera Williamsii* Standley, in Macbride, Field Mus. Bot. 13, pt. 2: 1135. 1938.

Herbaceous to subfrutescent erect plants, 2–3 ft. high, on sea cliffs, Puntilla, Salinas (11,317) and Punta Centinela (11,407), or becoming a scandent thick-stemmed shrub to 10 ft. high in low ground between Ancon and Santa Elena (11,186); summit of Chanduy Hills, 1800 ft. (11,045); stream bed, vic. La Brea, Piura, Peru (H&S 11,580). This

Plate 8.—Fig. 1. *Alternanthera ficoidea, March* 878, Jamaica; and to right, *A. flavicoma, Stewart* 1386, Galapagos Islands.—Fig. 2. *Alternanthera ficoidea, Stewart* 1396, Galapagos Islands; and *March* 1077, Jamaica.


1
2
Alternanthera echinocephala (Mart.) Christopherson
PLANTS OF THE
ASTOR EXPEDITION, 1930
GALAPAGOS ISLANDS
Volaintsera echinocephala
(Mart. f.) Hog.-
Stem woody at base,
sometimes 2 to 3 in. in diameter.
Height 5 to 6 ft; bark gray, not
exfoliating. Harsh, common near
shore.
Alt. 100 ft.
Indefatigable Island
April 6, 1930
H. A. Beaman
Alternanthera echinocephala (Mart.)
Christopherson
Alternanthera echinocephala
(Gaudich.) Urban
Smith of Santa Clara, Peru
July 6 1911
Alternanthera echinocephala
(Mart.) Hemsl.
Alternanthera sessilis
(L.) DC.
Alternanthera sessilis
(L.) DC.
Alternanthera sessilis
(L.) DC

variable species was originally described from Guayaquil. The leaf measurement given by Bentham for the typical plant with lanceolate-acuminate leaves was about 2.5–5 × 1–2 cm. (in var. *glomerata* the leaves were larger and lanceolate to ovate), but in the unusually flaccid plant represented by no. 11,580, the leaves are as much as 6 cm. broad. Pubescence varies in different specimens from a dense white subappressed covering, especially noticeable on the peduncles, to instances where the leaf surfaces have only a sparse and almost imperceptible appressed cinereous pubescence. In no. 11,317, for example, the leaf-pubescence is less than in *Terry* 1388 (*A. Williamsii*) from Panama. The filaments of *A. pubiflora* are expanded and flattened at the base, and as in the staminodia, the cells have thickened walls. *A. pubiflora* is close to *A. echinocephala,* which differs only in the longer staminal tube and usually less pubescent perianth, and sometimes larger anthers [7 mm. as compared with 5 mm.]. But *B. pubiflora* is also in the Galapagos Islands (*Stewart* 1379 and 1380 from Hood Island); and Suessenguth (l.c.) has cited *A. Williamsii* (*Schimpff* 183) from the Galapagos Islands.

I have seen specimens of *A. pubiflora* from the region of Arequipa, Peru (*Pennell* 13,131 and *Narzal* 2026). These are more pubescent and evidently of smaller stature than the Ecuadorean material. The species extends northward along the coast and lower Andean slope to Ecuador, where it is noted by Suessenguth (as *A. Williamsii*) from Puná Island (*Eggers* 14,771) and El Recreo (*Eggers* 14,814). By Standley, in Macbride, Field Mus. Bot. 13, pt. 2: 507. 1937, *A. pubiflora* is said to extend from Bolivia and Chile to Colombia. *A. Williamsii* is known from Panama and the southwest corner of Guatemala (Pl. 6, fig. 3; Pl. 7, fig. 4a).

92. A. PUNGENS HBK., Nov. Gen. & Sp. 2: 206. 1817; Standley, in Macbride, Field Mus. Bot. 13, pt. 2: 506. 1937. *A. achyrantha* R.Br. var. *pungens* Suessenguth, Lilloa 4: 125. 1939. A weedy prostrate plant of coastal sands and roadsides, Salinas (11,421 & 11,326). The perianth bracts are sharp as a needle and readily penetrate the skin. *A. pungens* was originally described from the Maypuri Falls of the Orinoco, but is widely distributed from Bolivia and Argentina to Mexico and the West Indies, according to Standley (l.c.). From *A. repens* it differs chiefly in the glabrous sepals. Andersson got *A. pungens* on Puná Island (according to R.E. Fries, Ark. f. Bot. 16, no. 12: 17. 1921, who cites localities for its occurrence in Bolivia, Argentina, and Brazil).

93. A. RUGULOSA (Rob.) Howell, Proc. Calif. Acad. Sci. ser. 4, 21: 111. 1933. *Telanthera rugulosa* Rob., Proc. Amer. Acad. 38: 139. 1902. This species, which has the habit of a low tree in the Galapagos Islands, was thought to be confined to Chatham Island. It has large recurved bracts in the inflorescence, and is in this respect quite different from other species of *Alternanthera.* I cannot distinguish Galapagos material, either by inflorescence, leaves or stems, from a collection made by *Worth & Morrison,* no. 15,681, 14 km. south of Chala, Dept. Arequipa, Peru (cf. Pl. 9, fig. 2). The Peruvian collection was from a low bush, but there is a well-known tendency for insular plants to become shrubby or tree-like.

94. A. TRUXILLENSIS HBK. Nov. Gen. & Sp. 2: 206. 1818. A slender erect plant, silvery-pubescent at least on the younger growth, with rather thin leaves. On river gravels, near La Brea, Peru (H&S 11,594 & 11,622) (Pl. 6, fig. 4) and *O.B. Horton* 11,577; Piura, *Ball,* April 13, 1882 (G); Talara, *Haught* 54. *A. truxillensis* was described from the coast at Truxillo. A photograph in the Gray Herbarium shows a relatively slender plant of this localized species, with inflorescence at the apex of the plant and partially pedunculate. The white shining perianth of *A. truxillensis* is somewhat purplish tinged, twice as long as the bracts, and characterized by short stellate white hairs which scarcely project beyond the segment-grooves. The stellate hairs on the leaves have soft branches, all of relatively the same size. *A. truxillensis* has much longer anthers and shorter filaments than *A. halimifolia;* the filaments are terete, brownish, and of firmer texture than the staminodia.

95. A. VESTITA (Ands.) Howell, Proc. Calif. Acad. Sci. ser. 4, 21: 108. 1933. *Telanthera vestita* Andersson, Eugenie Bot. [Kgl. Sv. Vet. Akad. Handl. 1853]: 169. 1855. This has always been considered as an endemic species of the Galapagos Islands, but I can find no difference whatever in the inflorescence, leaves, or peculiar stem pubescence to separate it from *Jaffuel* 1650 (G), collected at Arica, Chile, Nov. 3, 1931 (cf. Pl. 10, fig. 2).

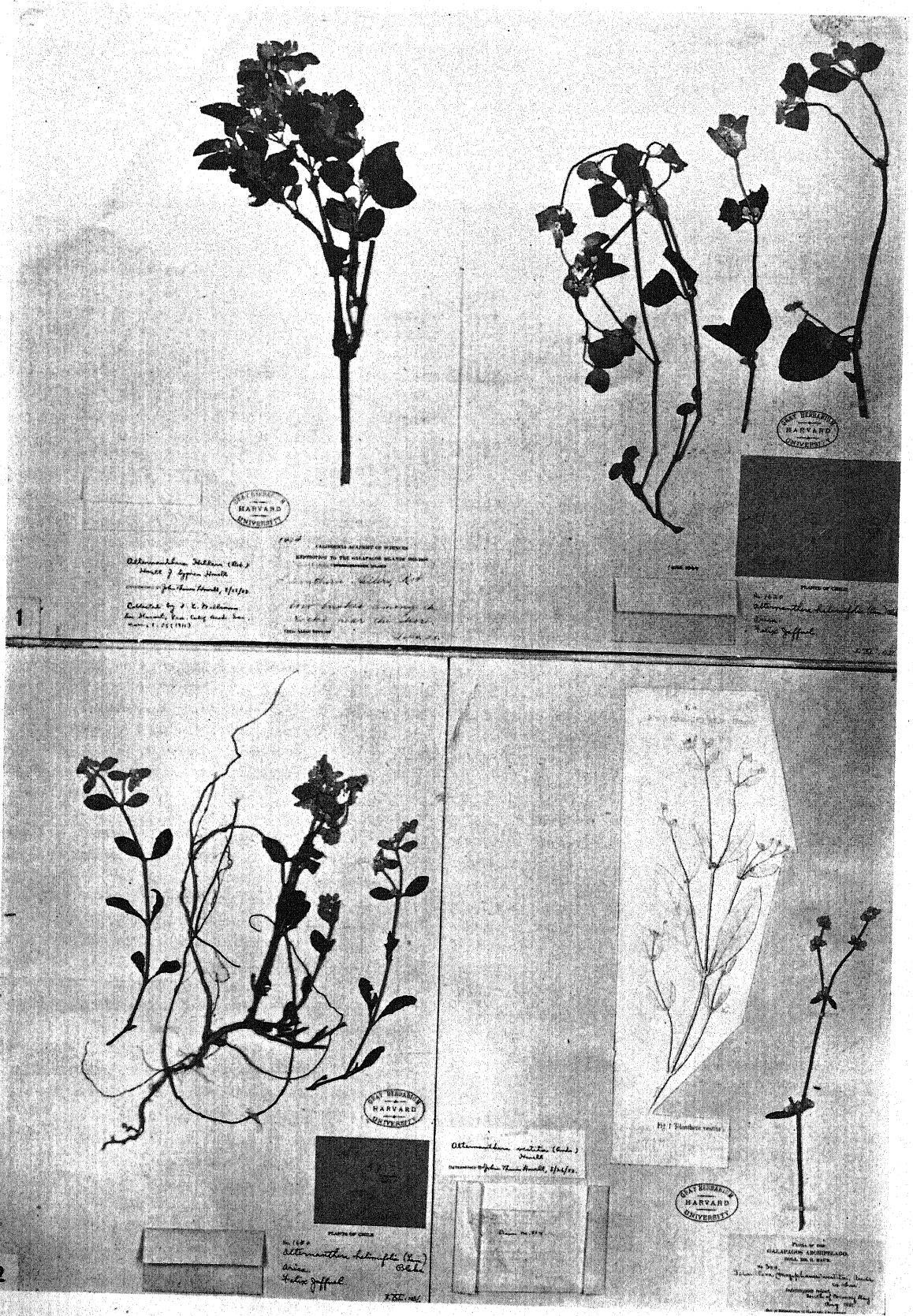
96. AMARANTHUS GRACILIS Desf., Tabl. Bot. 43. 1804. Waste ground at the seashore, Salinas (11,072). A common tropical weed, also in the Galapagos Islands.

97. A. HAUGHTII Standley in Field Mus. Bot. 11: 149. 1936, and 13: 486. 1937. A weedy species with linear leaves prominent in the inflorescence, broadly spathulate perianth segments, 2 to 3 stamens, and a circumscissile capsule, the upper portion of which is truncate and surmounted by a narrowed tridentate apex. This was collected in waste places and adjacent sandy fields at Salinas (11,280), and represents a species previously known only from the Pariñas Valley (*Haught*) and Talara (*I.M. Johnston* 3509) in Peru.

98. A. SPINOSUS L. Sp. Pl. 991. 1753. Roadside weed, Salinas (11,239). Common in the tropics and known from the Galapagos Islands.

99. A. aff. URCEOLATUS Bentham, Bot. Sulphur 158. 1846. Annual with lanceolate to ovate leaves, and with axillary to spicate inflorescence, on dry

Plate 9.—Fig. 1. *Alternanthera echinocepala, Rose* 18, 622, Peru; and *Svenson* no. 4, Galapagos Islands.—Fig. 2. *Alternanthera rugulosa, Worth & Morrison* 15, 681, Peru; and *Stewart* 1414, Galapagos Islands.


1
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GRAY HERBARIUM HARVARD UNIVERSITY
PLANTS OF GREECE
GALAPAGOS ARCHIPELAGO

soil on the Puntilla, Salinas (11,131) and in river gravels near La Brea, Piura, Peru (H&S 11,599). These collections are the same as *O.B. Horton* 11,553 (G) from Talara, and identical with a collection which I made on Black Beach (no. 152) on Charles Island in the Galapagos. It is quite evident that a species of the "squamulatus" group is common to the Galapagos Islands and the dry coastal region of Ecuador and northern Peru. Complications arise from the fact that no recent account of an authentic specimen of *A. urceolatus* has been published. The type, described as having five stamens, came from Guayaquil. My collections have consistently 3 stamens and a non-circumscissile capsule (a distinguishing feature of *Amblogyna* and *Scleropus* as treated by Moquin and by Andersson). This group distinction does not hold, since in *A. Haughtii* the capsule is circumscissile, though the sepals are urceolate. This is also true of some Galapagos specimens, such as *Baur* 390 from Jervis Island (named *A. squamulatus* by Suessenguth), which are exceedingly close to *A. Pringlei* of southwestern United States and Mexico.

The problem is further complicated by *A. quitensis* HBK., described from the Guallabamba River near Quito. This species is cited from Albemarle, Charles, and Indefatigable Islands by Howell, Proc. Calif. Acad. ser. 4, 21: 90. 1933, and also by Thellung in Ascherson & Graebner, Syn. 5, pt. 1: 253. 1914 (*Andersson* no. 61, in part). As a weed it is occasional in Europe; in South America it extends to Argentina and southern Brazil. The Galapagos plants are referred to a form with short bracts not longer than the perianth (cf. Thellung, p. 252), but differentiated from *A. dubius* Mart., by the strongly pubescent stem. Schinz in Engler, Pflanzenf. 16c: 38. 1934, describes the sepals of *A. quitensis* as "spathulate, stumpff oder gestutzt," and the illustration by Sandor, Magyar Bot. Lap. 13: 62. 1914, is certainly not far from that of *Amblogyna urceolata* Andersson (*Amaranthus Anderssoni* Howell, op. cit., p. 95). The Andean plant has recently been treated as *A. hybridus* L. var. *quitensis* (HBK.) Covas, Darwiniana 5: 336, fig. 3. 1941.

100. CELOSIA VIRGATA Jacq. Coll. Bot. 2: 279. 1788; Standley, Field Mus. Bot. 13, pt. 2: 481. 1937. Plants about 1 m. high with inconspicuous green flowers in panicles, in forest, Manglaralto (11,648). Peru to the West Indies and southern Mexico. Var. *paniculata* Suessenguth, Rep. Spec. Nov. 39: 5. 1935, is cited from El Recreo, Ecuador (*Eggers* 15,759).

101. FROELICHIA INTERRUPTA (L.) Moq. in DC. Prod. 13, pt. 2: 421. 1849; Fawcett & Rendle, Fl. Jam. 3, pt. 1: 137, fig. 44. 1914; Standley, Field Mus. Bot. 13, pt. 2: 489. 1937. *Gomphrena interrupta* L. Sp. Pl. 224. 1753. *F. tomentosa* Moq. in DC. Prod. 13, pt. 2: 421. 1849.

Open ground on ridge north of Santa Elena (11,145) and on seacliffs, Puntilla, Salinas (11,122). This plant varies greatly in height and in amount of pubescence, and small specimens look more like a *Plantago* than a member of the Amaranthaceae. This immature material is possibly *F. tomentosa* var. *angustialata* Suessenguth, Rep. Spec. Nov. 35: 308. 1934, noted from Uruguay, and represented by *Spruce* 6524 from Chanduy, Ecuador. A widespread species from Texas to Chile and Paraguay.

102. IRENELLA CHRYSOTRICHA Suessenguth, Rep. Spec. Nov. 35: 318. 1934. An inconspicuous herb with minute green flowers, Manglaralto (11,503). The leaves have a peculiar clavate-appressed pubescence. This single species forms the genus *Irenella*, cited by Suessenguth from five collections in coastal Ecuador, from Manabi Province (*Eggers* 14,833) to Balao (*Eggers* 14,145). It was collected by Schimpff in southern Babahoyo (no. 304) and by Holmgren at San Ignacio (no. 57). The citations state that it is a plant of shaded woods and cultivated places. At Manglaralto it was growing intermingled with various tropical weeds in a plantation area which has some precipitation throughout the year.

103. IRESINE CELOSIA L. Syst. Nat. ed. 10: 1291. 1759; Standley, Field Mus. Bot. 13, pt. 2: 515. 1937. Grassy summit of Cerro Prieto, 2500 ft. (H&S 11,342). A widely distributed weedy plant, common in the tropics.

104. I. EDMONSTONEI Hook. f., Trans. Linn. Soc. 20: 190. 1847; Howell, Proc. Calif. Acad. 4th ser. 21: 96. 1933. Herbaceous plants 3 ft. high, with spreading branches and flocculent greenish-brown flowers, summit of Chanduy Hills, 1800 ft. (11,063). This species was described from Darwin's collection on Charles Island in the Galapagos, and has been otherwise known only from an old collection by Edmonstone from Guayaquil. The latter collection, in the Gray Herbarium, agrees entirely with no. 11,063. It is probable that collections such as *Hitchcock* 20,118 (G) from between Salinas and Guayaquil, and *Hitchcock* 19,970 from Prov. Guayas, which have been labeled as *I. angustifolia*, are really *I. Edmonstonei*. *I. angustifolia* Euphrén, as represented by *Fredholm* 4405 from Porto Rico and my no. 431 from Barro Colorado, Panama, has acuminate perianth (ca. 1.5 mm. long) and bracts; *I. Edmonstonei* has obtuse perianth segments only 1.0 mm. long, acute bracts, and shiny black seeds 0.75 mm. wide.

NYCTAGINACEAE

105. ALLIONIA INCARNATA L. Syst. Nat. ed. 10. 890. 1759; Standley, Field Mus. Bot. 13, pt. 2: 535. 1937. *A. malacoides* Benth., Bot. Sulphur 44, 1844, sec. DC. Prod. 13, pt. 2: 434. 1849. A prostrate plant with minute purplish flowers enclosed in bracts, on open headlands exposed to salt spray,

Plate 10.—Fig. 1. *Alternanthera Helleri, Stewart* 1404, Galapagos Islands; and *Jaffuel* 1620, Chile.—Fig. 2. *Alternanthera vestita, Jaffuel* 1650, Chile; and *Baur* 304, Galapagos Islands.

Puntilla, Salinas (11,086). All the plants appear to be unusually pubescent annuals. The species, which ranges from Chile to southwestern United States, was based on Loefling's collection from Cumaná, on the coast of Venezuela. According to Heimerl, Oesterr. Bot. Zeitschr. 56: 250. 1906, the clavate glands of the fruit attach themselves to the adjacent bract, forming a structure easily dispersed by winds. It is unknown in the Galapagos.

106. BOERHAAVIA CARIBAEA Jacq., Obs. Bot. 4: 5. 1771; Standley, in Macbride, Field Mus. Bot. 13, pt. 2, 537. 1937. *B. viscosa* Lag. & Rodr., Anal. Cienc. Nat. 4: 256. 1801. A sprawling viscid plant with minute purplish flowers, Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,589). This plant closely resembles *B. coccinea* but the inflorescence branches are strongly glandular, and it is the same as my collection from Charles Island (no. 23) in the Galapagos Islands. Extends from southern United States and the West Indies to Peru.

107. B. COCCINEA Mill. var. LEIOCARPA (Heimerl) Standley, Field Mus. Bot. 11: 108. 1931. *B. paniculata* f. *leiocarpa* Heimerl, Oesterr. Bot. Zeitschr. 56: 252. 1906. Abundant on exposed headlands on the Puntilla, Salinas (11,125), apparently a much more robust plant than *B. caribaea*. The form with glabrous anthocarps ranges, according to Heimerl, along the southern border of the range of the species, from Uruguay to Argentina; it is also known from Huanuco, Peru (Macbride, Field Mus. Bot. 13, pt. 2: 538. 1937).

108. B. ERECTA L. Sp. Pl. 3. 1753; Standley, in Macbride, Field Mus. Bot. 13, pt. 2: 538. 1937. An abundant erect species with pale pink flowers in sandy soil, Salinas (11,159 and 11,204); dry rocks, Punta Centinela (11,393); Puntilla, Salinas (11,320); river gravels, near La Brea, Piura, Peru (H&S 11,557). The anthocarps vary from truncate to acute. In Peru this widely-distributed weed seems to be confined to the northwest coastal area (cf. Macbride). My collections do not differ at all from those of the Galapagos Islands and the West Indies. Widely distributed in the tropics. Mr. Hunter tells me that it is occasionally used in preparing a fatal mucilaginous drink.

109. B. TUBEROSA Lam., Ill. Gen. 1: 10. 1791; Killip, Smithson. Misc. Coll. 98, no. 8: 2. 1939. *Commicarpus tuberosus* Standley, Contrib. U.S. Nat. Herb. 18: 101. 1916 and in Macbride, Field Mus. Bot. 13, pt. 2: 539. 1937.

On disintegrated granitic rock, head of Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,526). This plant is cited by Standley from Talara (*Haught* 100) and from Dept. Lima (Chosica and Corcona). I saw no trace of it on the coast of Ecuador, but it is one of the most abundant species in the Galapagos Islands where it has usually been identified as *B. scandens*. According to Lamarck, Encyc. 5: 57. 1804, the tuberous roots are edible.

110. B. VERBENACEA Killip, Jour. Wash. Acad. Sci. 16: 567. 1926; Standley, in Macbride, Field Mus. Bot. 13, pt. 2: 538. 1937. Prostrate on sands back of Negritos, Piura, Peru (H&S 11,607). A species with racemose inflorescence, apparently confined to the Piura region (collections by Haught and by Weberbauer).

111. CRYPTOCARPUS PYRIFORMIS HBK. Nov. Gen. & Sp. 2: 188, t. 124. 1817; Standley, in Macbride, Field Mus. Bot. 13, pt. 2: 528. 1937. Dunes, Punta Centinela (11,408). Also in the dunes at Salinas, and in the Amotape Hills, Peru, at the head of Quebrada Mogollon. Due to its underground shoots it is difficult to eradicate once it has become established. This is also one of the most characteristic coastal plants of the Galapagos Islands. It is known in Peru from Cajamarca, Piura, and Tumbez.

112. MIRABILIS VIOLACEA (L.) Heimerl, Beitr. Syst. Nyctag. 23. 1897; Standley, Field Mus. Bot. 11: 112. 1931. *Allionia violacea* L. Syst. Nat. ed. 10: 890. 1759. A sprawling essentially glabrous herb with conspicuous pink to purple flowers, in thickets at west base of Chanduy Hills (11,054). This plant, known from southern Mexico to Ecuador, was based on Loefling's collection from Cumaná, Venezuela. It is somewhat weedy and, like *M. prostrata,* extends up into the Andes. Eggers got it at El Recreo and Pavon [or Tafalla] at Guayaquil (cf. DC. Prod. 13, pt. 2: 432. 1849).

PHYTOLACCACEAE

113. ACHATOCARPUS PUBESCENS C.H. Wright, Kew Bull. 1906: 6. 1906; Heimerl in Engler, Pflanzenfam. 16c: 177, fig. 82. 1834; Macbride, Field Mus. Bot. 13, pt. 2: 547. 1937. *A. mollis* H. Walter, Pflanzenr. IV 83: 136, fig. 40. 1909.

Rounded trees about 12 ft. in height, with apiculate leaf-blades up to 7 cm. in length, and a few scattered spines on the branches, Punta Ayangue (11,362); scattered trees 1 ft. or more in diameter at 1000 ft. on Chanduy Hills (11,008)—the largest tree on these hills. The branches in 11,008 are conspicuously spiny, and the obtuse to emarginate leaves much smaller (to 4.5 cm. long), but there is no doubt that both collections belong to the same species. By Heimerl, the genus is treated as one of the two forming the Achatocarpaceae. Hauman-Merck, Anales Mus. Hist. Nat. Buenos Aires 24: 502. 1913, thinks that *A. mollis* is only a large-leaved *A. praecox* Griseb. The Ecuadorean plant is certainly variable, and if Hauman-Merck is correct, the species would appear to extend from Argentina across to Ecuador. *A. praecox* is known from Bolivia and Peru (cf. Heimerl, l.c.). *A. pubescens* was based on *Jameson* 528 from Guayaquil, a collection which Schinz & Autran, in their treatment of the genus in Bull. Herb. Boiss. 1: 6. 1893, consider to be identical with *A. praecox*. The other collections of *A. pubescens* which I have seen are *Eggers* 14,936 and 15,503 (G) from El Recreo, Ecuador, and *Weberbauer* 7693 (G) from 200–300 m. alt. near Tumbez, Peru. Mr. Hunter tells me that the trunk of this tree often becomes hollow

and serves as a hiding place for the fer-de-lance (equis), a snake which is abundant in western Ecuador.

AIZOACEAE

114. MOLLUGO CERVIANA (L.) Seringe in DC. Prod. 1: 392. 1824; Howell, Proc. Calif. Acad. Sci. ser. 4, 21: 15. 1933. *Pharnaceum Cerviana* L. Sp. Pl. 1: 272. 1753. A very small weedy species with almost filiform glaucous leaves, on dunes, Salinas (11,189); Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,572). Of wide distribution in the Old World, and occasional in the New World tropics. It was obtained by Howell on several of the Galapagos Islands.

115. M. VERTICILLATA L. Sp. Pl. 89. 1753; Fawcett & Rendle, Fl. Jam. 3, pt. 1: 164, fig. 60. 1914. A variable annual with verticillate leaves, sea cliffs, Puntilla, Salinas (11,085); sandy flats, Salinas (11,156); river gravels near La Brea, Peru (H&S 11,561). Widespread weed in temperate and tropical America; not known from the Galapagos Islands.

116. SESUVIUM PORTULACASTRUM L. Syst. ed. 10: 1058. 1759; Fawcett & Rendle, Fl. Jam. 3, pt. 1: 166, fig. 61. 1914. Succulent plant of salt flats and beaches, Salinas (11,196). Tropics of both hemispheres; known from the Galapagos Islands.

117. TRIANTHEMA PORTULACASTRUM L. Sp. Pl. 233. 1753; Fawcett & Rendle, Fl. Jam. 3, pt. 1: 167, fig. 62. 1914. Prostrate annual with pale purplish flowers, on pathways, ridge north of Santa Elena (11,146). Seashores of Old World and New World tropics, and known from the Galapagos Islands.

PORTULACACEAE

118. PORTULACA HAUGHTII Macbride, Field Mus. Bot. 11: 21. 1931 and 13, pt. 2: 564. 1937. A yellow-flowered species with short linear leaves, sea cliffs, Puntilla, Salinas (11,218); sandy roadsides, Salinas (11,240); river gravels near La Brea, Peru (H&S 11,576). Previously known only from Piura, Peru (*Haught* F 180).

119. P. OLERACEA L. Sp. Pl. 445. 1753; Fawcett & Rendle, Fl. Jam. 3, pt. 1: 169, fig. 63. 1914. Sandy roadsides, Salinas (11,098). A weed in gardens and waste land nearly throughout the world.

120. TALINUM PANICULATUM (Jacq.) Gaertn. Fruct. 2: 219. 1791; Macbride, Field Mus. Bot. 13, pt. 2: 567. 1937. *T. patens* (Jacq.) Willd., Sp. Pl. 2: 863. 1800. *Portulaca paniculata* Jacq., Enum. 22. 1760. Purple-flowered herb 2–3 ft. high with long filiform peduncles, along forest pathways, Chanduy Hills, 1200 ft. (11,020); Manglaralto (11,497). This plant is of wide distribution in the American tropics from Florida southward. It is quite different from *T. crassifolia* of China and Formosa, a species with congested inflorescence frequently cultivated under the name of *T. patens*.

121. T. TRIANGULARE (Jacq.) Willd., Sp. Pl. 2: 862. 1800. *Portulaca triangularis* Jacq., Enum. Pl. Carib. 22, 1760. Abundant low plants on beaches and sea-cliffs, and frequently inland. Manglaralto (11,504); Punta Centinela (11,404); Santa Elena (11,135); Chanduy Hills (11,019); Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,512). The flowers range from purple to nearly white. According to Macbride, Fl. Peru [Field Mus. Bot. 13] pt. 2: 567. 1937, the plant is sometimes cooked and eaten as a vegetable. The species is widely distributed in tropical America, but is not known from the Galapagos Islands.

BASELLACEAE

122. BOUSSINGAULTIA FILIFORMIS (Moq.) Hauman, Anal. Mus. Hist. Nat. Buenos Aires 33: 352. 1925; Macbride, Field Mus. Bot. 13, pt. 2: 577. 1937. *Tandonia filiformis* Moquin in DC. Prod. 13, pt. 2: 227. 1849. A twining vine with minute white flowers (petals 1.4 mm. long) in long simple or bifurcate spikes, near summit, Chanduy Hills, 1600 ft. (11,064).

The genus, which centers in the Ecuadorean region, has been worked out in a provisional manner by Hauman (op. cit.) who has assigned to *B. ramosa* the Darwin collection from the Galapagos Islands previously referred to *B. baselloides* (cf. pp. 348 and 352). *B. ramosa* is otherwise known only from Guatemala, Costa Rica, and Mexico, except for the large-leaved var. *boliviensis* Hauman (op. cit., p. 353), with lax racemes and leaves 6 × 4.5 cm. described on the basis of *Mandon* 1027 from Sorata, Bolivia (alt. 2600 m.). This variety has perfect flowers and Hauman suspects that it may be a distinct species. No. 11,064 has much in common with *B. ramosa* (Moq.) Hemsley, as represented by *Pringle* 6627 from Morelos, Mexico, but the resemblance is largely superficial, my Ecuadorean collection differing in the large, very thin leaves with blades reaching 9 × 7 cm., and the spikes more slender and sinuous, with looser flowers. The petals are shorter (1.4 mm. long) as compared with those of Pringle's collection (1.7 mm. long) and broader. Both petals and sepals have a more coriacious texture in the Ecuadorean plant, and the sepal-base has projecting, almost spinose, opaque cells, as compared with the flat transparent reticulum in the Mexican. Furthermore, the trigonous-capitate stigma is more compact and lacks the minute ciliation seen in the Mexican plant. *B. filiformis* was known previously only from a *Ruiz & Pavon* collection from Peru (without locality).

NYMPHAECEAE

123. NYMPHAEA AMPLA (Salisb.) DC. var. PULCHELLA (DC.) Caspary in Mart., Fl. Bras. 4, pt. 2: 159, t. 30, f. 2–10. 1878; Conard, The Waterlilies, Carnegie Publ. no. 4: 136. 1905; Fawcett & Rendle, Fl. Jam. 3 pt. 1: 184. 1914. A waterlily with white flowers, pools south of Ancon (11,058, det. Conard). *N. ampla* is widely distributed in tropical America. Var. *pulchella* was based on a Pavon collection from Guayaquil; it extends from 2° to 23° in South America and is also known from the West Indies.

MENISPERMACEAE

124. CISSAMPELOS PAREIRA L. Sp. Pl. 1031. 1753; Fawcett & Rendle, Fl. Jam. 3, pt. 1: 193, fig. 79. 1914; Macbride, Field Mus. Bot. 13, pt. 2: 684. 1938. Climbing over bushes, Manglaralto (11,464). The specimens have peltate leaves, and greatly resemble plants of the Galapagos Islands, except that they are much less pubescent. *C. Pareira* is found in the tropics of both hemispheres.

CAPPARIDACEAE

125. CAPPARIS ANGULATA R.&P. Fl. Peruv. 4: pl. 431. 1802; Macbride, Field Mus. Bot. 13, pt. 2: 998. 1938. *C. scabrida* HBK. Nov. Gen. & Sp. 5: 95. 1821; Weberbauer, Veg. der Erde 12: 153, fig. 14. 1911.

Coarse-leaved trees 15 ft. high along railroad between Progresso and Baños San Vicente (11,501); dwarfed rounded trees, Quebrada Ancha, near La Brea, Peru (H&S 11,597). The first collection cited, being from a more mesophytic area, lacks the scabrous character of the upper leaf surface so characteristic of the Peruvian collections. The type came from Guayaquil. The species is also known from Negritos (*Haught*), Paita, and Lambayeque, in Peru.

126. C. CORDATA R.&P., Fl. Peruv. 4: pl. 432. 1802; Macbride, Field Mus. Bot. 13, pt. 2: 999. 1938. A single small shrub overhanging the sea on the north side of the Puntilla, Salinas (11,232); abundant shrubs to small spreading trees, Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,595). This species is characterized by small cordate leaves. The original collection came from Guayaquil. In Peru the species extends to Paita, Lambayaque, Patapo (*West* 3581), Truxillo, and Yaután (alt. 2000 ft., *Macbride & Featherstone* 2568). According to Macbride, it reaches Bolivia.

127. C. aff. MOLLIS HBK., Nov. Gen. & Sp. 5: 88. 1821; Macbride, l.c., p. 1001. Small trees with elongate lanceolate leaves (average 6 × 1.5 cm.) which are obtuse to acuminate, and finely pubescent with simple hairs on both surfaces. North side of Puntilla, Salinas (11,252); sea cliffs, Punta Ayangue (11,365); base of Cerro Prieto, Piura, Peru (H&S 11,569). This is exceedingly close in appearance to the wholly glabrous *C. eucalyptifolia* Haught, Tropical Woods, no. 20: 31. 1929, which was described from the Amotape Hills, east of Talara (*Haught* in 1928) and from near La Brea (*Haught* F 96). *C. mollis* came from banks of the Macara R., Peru, near Olleras.

128. C. OVALIFOLIA R.&P., Fl. Peruv. 4: t. 432. 1802; Macbride, l.c., p. 1002. *C. avicennifolia* HBK., Nov. Gen. & Sp. 5: 94. 1821. An abundant shrub or small tree, stellately pubescent, in the rolling country from Santa Elena to Ancon (11,171). I collected it also on Punta Ayangue (11,343) and at Punta Centinela (11,410). The original collection came from Guayaquil (*Tafalla*); the HBK specimen from "ad littora maris Pacifici et circa Guayaquil." It is abundant also in Peru east of Talara, and extends to Truxillo and to Ica, 350 m. alt. (*Weberbauer* 7196).

LEGUMINOSAE

129. ACACIA FARNESIANA (L.) Willd., Sp. Pl. 4: 1083. 1806; Fawcett & Rendle, Fl. Jam. 4: 139, f. 43. 1920. *Mimosa Farnesiana* L. Sp. Pl. 521. 1753. Erect bushes with glaucous leaflets and dark-brown unconstricted pods, open fields, Salinas (11,205). Florida to Argentina, and in the Old World tropics; known from the Galapagos Islands.

130. A. HUARANGO Ruiz ex Macbride, Field Mus. Bot. 8: 90. 1930 and Fl. Peru, pt. 3: 78. 1943. This is the most abundant leguminous shrub in the vicinity of Salinas, where it forms spreading thickets generally less than a meter in height. The branches shelter innumerable green lizards, large and small, that retreat rapidly to burrows beneath the spiny growth. The yellow flowers have a strong odor, and the short-velvety brown pods are so deeply constricted that they resemble strings of beads. Since the season of bloom is prolonged, flowers and mature fruit are frequently found at the same time. Abundant on the inner dunes, Salinas (11,149); river gravels, Quebrada Ancha, near La Brea, Peru (H&S 11,588). The entire plant is covered with an abundant short strigose pubescence; leaves are 4–10 cm. long; pinnae (15–18 pairs) are 2 cm. long; leaflets (28–32 pairs) 1.5 mm. long at maturity. The calyx is 1.5 mm. long, and the corolla 2.0–2.2 mm. long. According to Macbride, *A. huarango* is common in central Peru, being known from Huanuco, Chosica, Pariñas Valley (*Haught* F 78), Trujillo, and Lima (*Gaudichaud*).

131. A. MACRACANTHA H&B. ex Willd., Sp. Pl. 4: 1080. 1806; Kunth, Mimos. t. 28. 1819; Bentham, Trans. Linn. Soc. 30: 500. 1875; Macbride, Fl. Peru, pt. 3: 80. 1943. *A. punctata* H&B. ex Willd., Sp. Pl. 4: 1084. 1806 (sec. Diels). *A. cochliacantha* H&B. ex Willd., Sp. Pl. 4: 1081. 1806; Kunth, Mimos. 93, t. 29. 1819.

This species was described from Guayaquil (as was also *A. cochliacantha*). The type is well matched by *Schimpff* 383 from western Guayaquil, (according to Diels, Fl. Ecuad. 92. 1937), a specimen with large flattened thorns 7–8 cm. long on the old growth; and with short spines only 2–3 mm. long on the newer growth. No pods were known from the original HBK. specimens. The *Schimpff* collection is identical with Haught 212 from the Pariñas Valley, Peru, where I saw it as a flat-topped tree with non-constricted pods, quite different in appearance from the shrubby *A. huarango*. Leaflets in the Schimpff and Haught collections are pubescent and 1.25–2.0 mm. long, and the specimens are indistinguishable from such Galapagos collections as *Von Hagen* 74c (Duncan I.) and 10 (Indefatigable I.), both issued as *A. tortuosa*. Christophersen, Nyt. Mag. Naturv. 70: 76. 1932, has described *Acacia Rorudiana* (based on a specimen from Indefatigable Island, but of wider distribution in the Galapagos Islands) which has the

pubescent rachis and leaves of *A. macracantha* of the Guayaquil area, but in which the leaves are shorter (0.6–0.8 mm. long). I suspect that *A. Rorudiana* represents a reduced form of the continental *A. macracantha,* perhaps an extreme of such material as *Von Hagen* 10, a collection with only 5–9 pairs of pinnae and hispid leaflets 1.5–2 mm. long. The calyx of *A. Rorudiana* is identical with that of *A. macracantha.*

Mimosa lutea Miller, Dict. ed. 8, no. 1768, based on a Houstoun manuscript polynomial without description, is said by Britten and Baker, Jour. Bot. 35: 226. 1897, to be from Jamaica. Fawcett & Rendle, Fl. Jamaica 4, pt. 2: 137. 1920, treat the Jamaican *A. lutea* as having leaflets in 20–30 pairs and 2–3 mm. long, and consider *A. macracantha* to be a synonym. But Britton & Killip, Ann. N. Y. Acad. Sci. 35: 139. 1936, limit the distribution of *A. macracantha* to Ecuador and Peru, which would appear to be correct.

A. tortuosa, as collected by me in the Galapagos Islands (no. 277) has 2–3 pairs of pinnae, and 4–9 pairs of acute glabrous leaflets (5–9 mm. long, and 1–3 mm. wide), and a dull brown pod (slightly constricted) with surface lines like those of *Prosopis.* This collection conforms with the large-leaved *A. insulae-Iacobi* Riley, Kew Bull. Misc. Inf. 1925. 220. 1925, but not to *A. tortuosa* as treated by Fawcett & Rendle, Fl. Jam. 4, pt. 2: 138. 1920: "pinnae in 2–8 pairs; leaflets in 10–20 pairs, 4–5 (–7) mm. long."

132. AESCHYNOMENE PLURIARTICULATA G. Don, Gen. Syst. 2: 284. 1832. Annual with cream-colored flowers with brown center, in dry fields, Salinas (11,329). The pods have 9–14 segments; some of the pods are smooth, while in others on the same plant the segments have raised scabrous centers. This difference between smooth and scabrous segments is the only note of differentiation between *A. pluriarticulata* (described as having 14–18 segments) and *A. scabra* Don (l.c.), both described from Lambert's herbarium as coming from Guayaquil. It is probable therefore that the two names are synonymous. Representative of *A. pluriarticulata* are *Hitchcock* 20,073 (June 21–24, 1923) from the Oil Camp between Salinas and Guayaquil; *Eggers* 14,775 from Puná Island; *Haught* 158 from the Pariñas Valley, Peru, and from 25 miles east of Cabo Blanco, Peru. Haught's no. 158 has been cited by Macbride, Fl. Peru, pt. 3: 444. 1943, as *A. indica* L.

133. CAESALPINIA BONDUCELLA (L.) Fleming in As. Res. 11: 159. 1810; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 93, fig. 32. 1920. *Guilandina Bonducella* L. Sp. Pl. ed. 2: 545. 1762. A single plant on the beach at Punta Ayangue (11,363); abundant on the sandy beach at Manglaralto (11,467). This prickly plant with yellow flowers is the source of the gray nickar nuts of the tropics which are sometimes cast ashore on the coast of Ireland and Scotland. I believe that these stations represent the southern limit of the plant on the west coast of South America, and consequently, together with associated plants (see introduction, the southern limit of the tropical strand flora. In the Galapagos Islands, the only recorded locality for this spectacular plant is at Villamil, Albemarle Island, where it was obtained by Baur and Stewart. Since the seeds float readily, the conclusion may be drawn that the coastal areas of the Galapagos Islands are in general too dry to maintain the plant.

134. C. CORYMBOSA Benth., Pl. Hartw. 117. 1839 and Bot. Sulphur 87. 1844. A thornless shrub to small spreading tree 10 ft. high. The fleshy pods are flattened, 5–8 cm. long, about 1.5 cm. broad, and nearly coal-black when mature. Ridge north of Santa Elena (11,368); near summit, Chanduy Hills, 1800 ft. (11,027). The type came from Guayaquil (*Hartweg* 651), and I have seen a photograph in the Gray Herbarium. Further specimens are from Duran, Ecuador (*Rose & Rose* 23,600) and from between Serrán and Salitral, Piura, Peru, 170–200 m. (*Weberbauer* 5994). Areschoug (op. cit., p. 141) cites it from Puná Island.

135. C. PULCHERRIMA (L.) Sw. Obs. 166. 1791; Macbride, Fl. Peru, pt. 3: 194. 1943. *Poinciana pulcherrima* L. Sp. Pl. 380. 1753. The "Pride of Barbados," grown in all tropical countries, is occasionally an escape from cultivation. Between Ancon and Santa Elena (11,188).

136. CASSIA NICTITANS L. Sp. Pl. 543. 1753. Erect annual with minute yellow flowers, in dry fields, Punta Centinela (11,264). This Ecuadorean plant is undoubtedly the species that occurs commonly in United States, and bears closest resemblance to robust specimens from Arkansas and Texas. The leaves reach 5.5 cm. long (with 8–12 pairs of obtuse leaflets 1.5 cm. long); the stipitate gland is 0.5–0.75 mm. wide; stipules prominently veined, 7–7.5 mm. long; petals 4–6 mm. long; pods 3.5 cm. long, 3.5–4 mm. wide, 12–14-seeded, prominently appressed-pubescent; stamens 5, 3–4 mm. long. It coincides well with the treatment of *C. nictitans* by Fawcett & Rendle, Fl. Jam. 4: pt. 2: 114. 1920. Andersson obtained it on Puná Island, but there is no report from the Galapagos. Superficially the resemblance to Porto Rican specimens, such as *Fredholm* 4269 and 4373, is close; but these collections have 9 stamens of varying size, and would therefore be placed under *Chamaecrista aeschinomene* (DC.) Greene, as treated by Britton & Wilson, Bot. Surv. Porto Rico and Virgin Islands 5: 368. 1923, or under *Cassia mimosoides* in Bentham's fundamental treatment of the group (Trans. Linn. Soc. 27: 579. 1871).

C. nictitans was illustrated and described in Hortus Cliffortianus, where the plant was grown from seeds derived from Virginia, and the description is clear: resembles *C. Chamaecrista,* but smaller and with small flowers borne singly; four of the petals are small, connivent, and persisting closed, but the lowest or fifth petal is four times larger, and constitutes a lower lip; the five stamens are small and ascending; leaves pinnate (12–14 pairs),

leaflets linear, gland subrotund on petiole below leaflets, black; stipules acute. It should be pointed out that in some material from eastern United States and likewise in 11,264 the fifth petal is scarcely larger than the others.

137. C. OCCIDENTALIS L. Sp. Pl. 377. 1753; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 104. 1920; Macbride, Fl. Peru, pt. 3: 175. 1943. Forming large stands at the upper margin of the beach, Manglaralto (11,466), apparently here reaching its southern limit on the coast. An abundant yellow-flowered weed, probably of American origin, in the tropics of both hemispheres.

138. C. OXYPHYLLA Kunth, Mimos. 129, t. 39: 1819; Bentham, Trans. Linn. Soc. 27: 521, t. 61. 1871. *C. Hartwegi* Benth., Pl. Hartw. 117. 1839. This yellow-flowered tree Mr. Kenward grew in his garden at Salinas (11,466a), having obtained it from the region east of Santa Elena. Mexico to Ecuador; *C. Hartwegi* came from Guayaquil.

139. CROTALARIA PUMILA Ortega, Hort. Bot. Matrit. Dec. 2: 23. 1797; Senn, Rhodora 41: 358. 1939. *C. puberula* Hook. f. Trans. Linn. Soc. 20: 225. 1847. Yellow-flowered annual in river gravels, Quebrada Ancha, near La Brea, Peru (H&S 11,571, det. Senn). The plants are lightly appressed-puberulent with lanceolate-oblong leaflets up to 3.5 cm. long, with 9–11-flowered racemes. The variant which I collected in the Galapagos Islands (no. 251) is much dwarfed, with emarginate obovate leaflets not exceeding 1 cm. in length and with 1–2-flowered racemes, and is evidently *C. puberula*. *C. setifera* DC., known from the Galapagos Islands, is synonymous with *C. incana* L. (cf. Senn, p. 350). Both of these species of *Crotalaria* are of wide distribution (Senn, p. 318): "*C. incana* L. is a widespread tropical weed, native to America but now common throughout the Old World tropics as well. *Crotalaria pumila* Ortega is another species which tends to become a weed and presents a wide range of variability."

140. DALEA LINNAEI Michx., Fl. Bor.-Am. 2: 57. 1803. *Psoralea Dalea* L. Sp. Pl. 1076. 1753. *Dalea Cliffortiana* Willd., Sp. Pl. 3: 1336. 1803. *Parosela Cliffortiana* Rose, Contrib. U.S. Nat. Herb. 10: 105. 1906. *Thornbera Dalea* Rydberg, N. Am. Fl. 24: 120. 1920.

Annuals to 3 ft. high in fields and dunes, Punta Centinela (11,266), the plants just coming into flower. The minute purple flowers are in elongate heads, and though the bracts have conspicuous resinous dots, they do not have the blackening seen in many Mexican specimens. This plant has exactly the structure of *Petalostemon corymbosum*, except that the stamens are 10 instead of 5; it was originally obtained by Houston from Vera Cruz, Mexico, and was grown independently by Phillip Miller, who listed the name *Psoralea annua* (in synonymy of *Psoralea Dalea*) in Gardener's Dict. ed. 8. 1768, a name which was revived as *Dalea annua* O. Kuntze, Rev. Gen. 178. 1891. The plant is abundant in Mexico, and is known from the dry coast of Venezuela.

141. DESMANTHUS VIRGATUS Willd., Sp. Pl. 4: 1047. 1806; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 131, fig. 41. 1920; Macbride, Fl. Peru, pt. 3: 98. 1943. *D. depressus* H&B. ex Willd., Sp. Pl. 4: 1046. 1806. Prostrate to erect plants, with flowers in small white heads, low ground north of Ancon (11,417). In this disposal of species I have followed Fawcett & Rendle who say, "Bentham retains *D. depressus* H&B. as a species distinct from *D. virgatus*. It seems to us merely a weak form of the species." *D. depressus* was described originally from Moche on the Peruvian coast and is known on the Galapagos Islands from collections extending back to Darwin's time. *D. virgatus* is widely dispersed from Florida southward.

142. DESMODIUM GLABRUM (Mill.) DC. Prod. 2: 388. 1825. *Hedysarum glabrum* Mill., Gard. Dict. ed. 8, no. 12. 1768. *Hedysarum molle* Vahl, Symb. 2: 83. 1791. Dry rocks, Punta Centinela (11,395); base of Cerro Prieto, Piura, Peru (H&S 11,554), a much smaller, less pubescent plant 1 ft. high. Dr. Robinson (p. 151) is of the opinion that this widely-distributed tropical plant has been recently introduced into the Galapagos Islands. The species occupies southern Mexico, Central America, most of the West Indies, and the northern third of South America (cf. Schubert in Macbride, Fl. Peru, pt. 3: 426. 1943).

143. D. PROCUMBENS (Mill.) Hitchcock, Rept. Missouri Bot. Gard. 4: 76. 1893. Sandy fields, Salinas (11,203), dwarf plants with stems only 10–16 cm. long; shaded rocks, Puntilla, Salinas (11,224), stems 5–6 dm. long. This is the typical plant as treated by Schubert, Contrib. Gray Herb. n. s. no. 129: 5, t. 1, fig. B. 1940, which extends from Mexico and the West Indies to Ecuador. The small specimens are similar to those found on the Galapagos Islands. The central branch is usually erect and the lateral branches procumbent. The mature flowers are very small, white, with the wings slightly pink-tinged; the pods are prominently spiral.

144. DIOCLEA LEHMANNI Diels, Bibl. Bot. Heft 116: 97. 1937, e descr. Coarse vine climbing over shrubs, with purple flowers 2–3 cm. long, Manglaralto (11,450). This plant belongs to section *Eudioclea*, with the 10 anthers of uniform size. The standard and keel, as described by Diels, are about 14 mm. long, but in no. 11,450 they may be as much as 2.5 cm. long. Flowers are variable in size and Diels may have described small-flowered material. *D. Lehmanni* was based on a collection from Naranjal, Ecuador; *Mille* 63 from rain forest, Boliche, Prov. Guayas, was also cited. No. 11,450 has leaves and flowers practically identical with those of *Rusby* 2141 from Bolivia, determined as the Brazilian *D. coriacea* Benth. From Guayaquil Bentham (Bot. Sulphur 85. 1844) described *D. guianensis* var. *velutina*, which appears (from description) to be more villous than no. 11,450.

145. ERYTHRINA VELUTINA Willd., Ges. Nat. Freunde Berlin Neue Schr. 3: 426. 1801; Krukoff, Brittonia 3: 326. 1939. *E. splendida* Diels, Bibl. Bot. [Fl. Ecuad.] 116: 96. 1937.

Spreading tree 20 feet high with spinescent branches, ridge north of Santa Elena (11,108) (Feb. 22, 1941, in fruit, and with only a few seeds remaining). The ovate-rhombic retuse leaflets are truncate at the base, 12–15 cm. long, and lightly stellate-pubescent; the venation, pubescence and texture of the leaves is exactly that of Galapagos specimens, such as *Chapin* 1127 and *Von Hagen* 100a from Indefatigable Island, through the leaflets in those specimens are only 2–6 cm. long. The seed in no. 11,108 is shiny scarlet throughout, 13 mm. long and 7 mm. wide, and lacks entirely the "broad black line extending from the hilum for approximately 3 mm. toward the chalazal end" (cf. Krukoff, l.c.). The stamens in Von Hagen 100a are only 4 cm. long, and the standard 22 mm. wide; in *E. splendida,* which came from 30 km. west of Guayaquil (flowering state only, without leaves, Sept. 29, 1933), the stamens are described as 5–6 cm. long and the standard as 5.0–5.5 cm. long and 15–28 mm. wide. The Galapagos specimens therefore appear in all respects to be a reduced form of the mainland species. *E. velutina* is widely distributed in the drier American tropics. "It is found in the West Indies and in northern Venezuela; on the west coast it reappears in Ecuador and the Galapagos Islands, whereas on the east coast it is known from the state of Ceara, Brazil, and practically throughout southern Brazil." . . . "In the West Indies and in Venezuela the species usually flowers in March or April, in Ecuador and in Brazil from July to November. The species is often planted for ornamental purposes" (Krukoff, p. 328–329).

146. GALACTEA VELUTINA Benth. in Tayl., Ann. Nat. Hist. 3: 437. 1839; Killip, Smithson. Miscel. Coll. 98, no. 8: 2. 1939. A slender vine with lavender flowers, in river gravels, Quebrada Ancha, near La Brea, Peru (H&S 11,627). As Killip has pointed out, the correct name for the Galapagos plant is *G. velutina,* as Andersson had previously named it, and not *G. Jussiaeana* var. *volubilis.* This Peruvian plant (identical with *Haught* 85 from Talara) has the same flowers and pods and the same type of pubescence, especially marked in the developing leaves, as in Galapagos collections. But the mature leaflets are much larger, reaching 8 × 5 cm. in no. 11,627 and frequently acute at the apex; whereas in Galapagos specimens they are about 3 × 1.5 cm. and slightly emarginate, though the seta terminating the midvein is of the same character in both. There is great variability in Galapagos material; some specimens, such as *Schimpff* 26 from Indefatigable Island, have even smaller leaflets (often not more than 1 cm. long) with rather sparse pubescence. *G. velutina* (cf. Hooker, Jour. Bot. 2: 59. 1840) was originally described from British Guiana (*Schomburgk* 649). The Peruvian plant, based on collections from Tumbez (*Weberbauer* 7733) and Piura (*Haught* 85), has been described as *G. Jussiaeana* var. *peruviana* Macbride, Fl. Peru 13, pt. 3: 333. 1943, since the foregoing notes were written.

147. GEOFFRAEA SPINOSA Jacq., Sel. Stirp. Amer. 207, t. 180, f. 62. 1763; Don, Syst. 2: 426. 1832. *Robinia striata* Willd., Sp. Pl. 3: 1132. 1803. *Geoffraea striata* Morong, Ann. N.Y. Acad. Sci. 7: 87. 1893; Stewart, Proc. Calif. Acad. ser. 4, 1: 74. 1911; Macbride, Fl. Peru, pt. 3: 255. 1943.

Rounded tree, with dark yellow flowers and drupe-like fruit resembling almonds, ridge north of Santa Elena (11,140). The species originally came from sandy woods near the seashore at Carthagena, Colombia, and is of wide distribution in dry tropics of South America. It is known from a single locality near an old habitation on Charles Island in the Galapagos.

148. HOFFMANNSEGGIA VISCOSA Hooker & Arnott, Bot. Miscel. 3: 209. 1833; Ball, Jour. Linn. Soc. 22: 150. 1887. *Caesalpinia viscosa* (R.&P.) Macbride, Fl. Peru 13, pt. 3: 197. 1943. A nearly prostrate, viscid-stemmed, somewhat shrubby plant with orange flowers, on the dunes at Negritos, Piura, Peru (H&S 11,611). It was collected by Haught at Talara, and ascends to 2300 m. at Apurimác in southern Peru (cf. Macbride). Commenting on this species, which was one of the 12 plants found at Payta in a dry season, Ball says "the labels of Cuming's collections were affixed without any close regard to topographical accuracy. It would seem as if but two labels were used for all of Cuming's plants collected on the west coast of tropical America: 'between 18 or 20 S. lat. and 10 N. lat.'—either that above quoted or 'Lima et Peruvia septentrionalis'" (fig. 20).

149. INDIGOFERA LEPTOSEPALA Nutt. ex Torr. & Gray, Fl. N. Am. 1: 298. 1838. Perennial with numerous prostrate branches and dull red flowers borne on peduncles exceeding the leaves, ridge north of Santa Elena (11,378); banks along railroad, La Libertad (11,293). This is one of the earliest plants to bloom in the rainy season. I collected it on Feb. 22 and saw it in bloom a week earlier, together with the yellow flowers of *Apodanthera biflora.* The specimens agree in all respects with those of *I. leptosepala,* which extends from Georgia to Kansas, Coahuila and Tamaulipas. The corollas were described by Torrey and Gray as "pale scarlet," and the length of the corolla is given by Rydberg as 10–11 mm., though in all the specimens that I have seen it is 9 mm. long, just as in the Ecuadorean specimens. No similar species is included by Burkart in his paper on the species of *Indigofera* of Argentina, Darwiniana 4: 145–178. 1942. Some of the duplicates were issued by me as *I. laxa* Ulbrich, Rep. Spec. Nov. 2: 4. 1906, from Cajamarca, Peru, which the Ecuadorean material resembles (as to photograph).

150. MIMOSA ALBIDA H&B. ex Willd., Sp. Pl. 4: 1030. 1806; Bentham in Hook. Jour. Bot. 4: 360.

1842; Britton & Killip, Ann. N. Y. Acad. Sci. 35: 150. 1936. A prickly somewhat shrubby plant, sometimes climbing, 2–4 ft. high, with bright pink flowers, and with strigose yellow to white pubescence of varying density. Among rocks, Puntilla, Salinas (11,222); hills north of Punta Carnera (11,302); dry slopes at base of Cerro Prieto, Amotape Hills, Peru (H&S 11,577).

The original collection came from maritime sands at Moche (cf. Kunth, Mimos. t. 1. 1819) near Lima, Peru. The leaflets were described by Willdenow as having short white pilae on each surface, but this spreading pubescence frequently disappears on the upper surface as the leaf matures. From the similar, but distinct, *M. floribunda* Willd. (described from Venezuela and extending north to Mexica (cf. *Pringle* 3970 from San Luis Potosi), *M. albida* differs in the pale, not dark green, upper leaf surface, less appressed pubescence on the lower leaf surface, and setose spreading pubescence of the pods. By Robinson, Proc. Am. Acad. 33: 310. 1898, where *M. floribunda* is treated as a variety of *M. albida, Pringle* 3970 is cited as representing typical *M. albida,* which is obviously not the case. In no. 11,222 the inflorescence becomes a terminal panicle; it undoubtedly represents the plant listed by Areschoug (p. 142) from Puná Island as *M. floribunda* var. *Willdenowii* DC. Prod. 2: 426. 1825. *M. Willdenowii* Poir., Encyc. Suppl. 1: 50. 1810, was a direct renaming of *M. floribunda* Willd., Sp. Pl. 4: 1031. 1806, a later homonym of *M. floribunda* Cels (1803). It is questionable whether the nearly glabrous specimens from the Ecuadorean Andes (cf. *Penland* 851 from Ibarra) represent *M. albida,* though Macbride has described the almost glabrous var. *erratica,* Bot. Field Mus. 8: 91. 1930, from the Lima region and northward in Peru. Typical *M. albida* I have seen from the following additional collections. Peru: Callao, *Wilkes Exped.* (US); Trujillo, *Killip & Smith* 21,518; Pariñas Valley, *Haught* 98; Cerro Viento, Paita, *Haught* 241. Ecuador: Guayaquil, *Rose* 22, 111; western Guayaquil, *Schimpff* 411 (B), as *M. floribunda;* Cerro Santa Ana, Guayaquil, *Jameson* 582 (April, 1846) (US); San Cristobal, Galapagos Islands, *Schimpff* 178 (B). In treating var. *erratica,* Macbride states, "The plant described here may not be constant in character, but it appears to be as distinct from *M. albida* as is *M. floribunda.* The latter typically is a much coarser species, more hairy and more abundantly flowered, but there are perplexing intermediate forms." Variations of *M. albida* have been more recently discussed by Macbride, Fl. Peru, pt. 3: 86–87. 1943.

151. Neptunia pubescens Benth., in Hook. London Jour. Bot. 4: 356. 1842; Macbride, Fl. Peru, pt. 3: 108. 1943. Elongate trailing plants with heads of yellow flowers in river gravels, Quebrada Ancha, near La Brea, Peru (H&S 11,612). Bentham (l.c.) based his species on *Cuming* 1027, from Lima, with the note "Very near *N. lutea.* The stipules are larger, the flower heads much smaller, and the stalk of the pod scarcely more than a line long." No. 11,612 is not only pubescent on stems and pods, but has small aculeate prominences which roughen the stems. Pods are 4–4.5 cm. long and 12-seeded. The two large acuminate bracts, situated one-third the distance up the peduncle, are of the same size and texture as the stipules. The seeds are not thin and flattened as in *N. lutea* (5.5 × 4.0 mm.) but are thickened (4.5 × 3.0 mm.) and exactly of the same size and consistency as those of *N. floridana* Small. In the Galapagos Islands occur glabrous plants with exactly this same large type of peduncle bracts, and exactly the same pods and seeds, except for reduced size, the pods being only 1.5–2.5 cm. long with 5–9 seeds or even smaller. The leaves are smaller, with only 7–10 pairs of leaflets on each pinna, and the pinnae only 1.0–1.5 cm. long. These Galapagos plants (such as my no. 225 from Indefatigable Island) grow in dry soil. I believe they represent dwarfed forms of the Peruvian *N. pubescens.* Certainly they have little to do with *N. plena* as exemplified by Miller's Ic. 182, or of Hooker in Bot. Mag. t. 4695, or of Fawcett & Rendle, Fl. Jam. 4, pt. 2: 130. 1920, who treat *N. plena* as "very like *N. oleracea* Lour. and perhaps not separable as a distinct species," and with "*Leaflets* 12–40 pairs. *Pod* larger (to 5 cm. l.), more erect. *Seeds* 5–20. Plant more terrestrial." *Neptunia pubescens* extends from Florida and Texas to Paraguay.

152. *Phaseolus lathyroides* L. Sp. Pl. ed. 2, 1018. 1763; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 65. 1920; Hassler, Candollea 1: 446. 1923. *P. semierectus* L. Mant. 1001. 1767; Stewart, Proc. Calif. Acad. Sci. ser. 4, 1: 76. 1911. *P. mollis* Hook f. Trans. Linn. Soc. 20: 228. 1847; Stewart, l.c.

Semi-erect annual with dark purple flowers, in river gravels, Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,578). In this collection the lateral leaflets tend to be slightly lobed, the stems are prominently retrorsely pubescent, and the pods are only 5 cm. long (Fawcett & Rendle, l.c., give the length of the pods as 8–10 cm.). However, the species is widespread and variable in the tropics of both hemispheres and this Peruvian collection is, except for somewhat stronger pubescence, the same as Galapagos collections (cf. my no. 172 from Charles Island) and specimens (cf. *Fredholm* 4319) from Porto Rico. No. 11,578 belongs to the broad-leaved var. *semierectus* (L.) Hassler (l.c.) and var. *genuinus* was cited by Hassler on the basis of Andersson's collection (as *P. cystoides* Zoll. & Mor.) from Charles Island, Galapagos. Whether these varietal distinctions are significant is questionable. *P. mollis,* described from the Galapagos Islands, is cited as a synonym of *P. semierectus* by Rock, Leg. Hawaii 217. 1920. The peduncles of 11,578 are retrorse pubescent, and the plant therefore perhaps represents *P. fraternus* Piper, Contrib. U.S. Nat. Herb. 22: 679. 1926.

153. P. peduncularis HBK. Nov. Gen. & Sp. 6: 447. 1824; Hassler, Candollea 1: 433. 1923. *Vigna*

peduncularis Fawcett & Rendle, Fl. Jam. 4, pt. 2: 68. 1920; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 5: 423. 1924.

Vine with bright purple flowers, climbing over bushes, Puntilla, Salinas (11,250). The flowers are variable in size but the standard in some of them reaches 2 cm. in length; thus the plant probably represents var. *genuinus* Hassler (p. 434), though it may well include var. *pusillus* Hassler (p. 436) described as having broad, thin membranaceous leaflets and small flowers only 1.5 cm. long, based in part on *Eggers* 14,636 from Balao, Ecuador. This variable species is widespread in the American tropics from Cuba to Peru and Paraguay. Fawcett & Rendle have transferred the species to *Vigna* since there is "no indication of the complete spiral twist of the keel, which seems to be the only character that differentiates the two genera." There is a strong partial twisting of the keel in the specimens which I collected; in the interest of stability of botanical nomenclature it seems that the plant could as well have been left in *Phaseolus* by Fawcett & Rendle, whose work in general is a model.

154. PITHECOLOBIUM CANDIDUM (HBK.) Benth. in Hooker, London Jour. Bot. 3: 201. 1844. *Inga candida* HBK. Nov. Gen. & Sp. 6: 299. 1824. Thorny bushes 5–8 ft. high, with tufts of white flowers followed by coiled pods, Puntilla, Salinas (11,111). This was originally described from Guayaquil and is apparently confined to the region. By Bentham, Trans. Linn. Soc. 30: 574. 1885, it is united with *P. excelsum* Mart. and cited as growing on the Magdalena River, but the account of Colombian species by Britton & Killip (Ann. N.Y. Acad. Sci. 35: 124–125. 1936) does not mention *P. candidum*. The calyx is 0.8 mm. long; the corolla is 4-lobed and 6–7 mm. long; the glabrous pods 1 cm. wide.

155. PROSOPIS JULIFLORA (Sw.) DC. Prod. 2: 447. 1825; Bentham, Trans. Linn. Soc. 30: 377. 1875; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 128. 1920 (in part); Burkart, Darwiniana 4: t. 22. 1940. *Mimosa juliflora* Sw. Prod. 85. 1788. *?Prosopis dulcis* Kunth, Mimos. 110. t. 34. 1822. *P. inermis* HBK., Nov. Gen. & Sp. 6: 307. 1825.

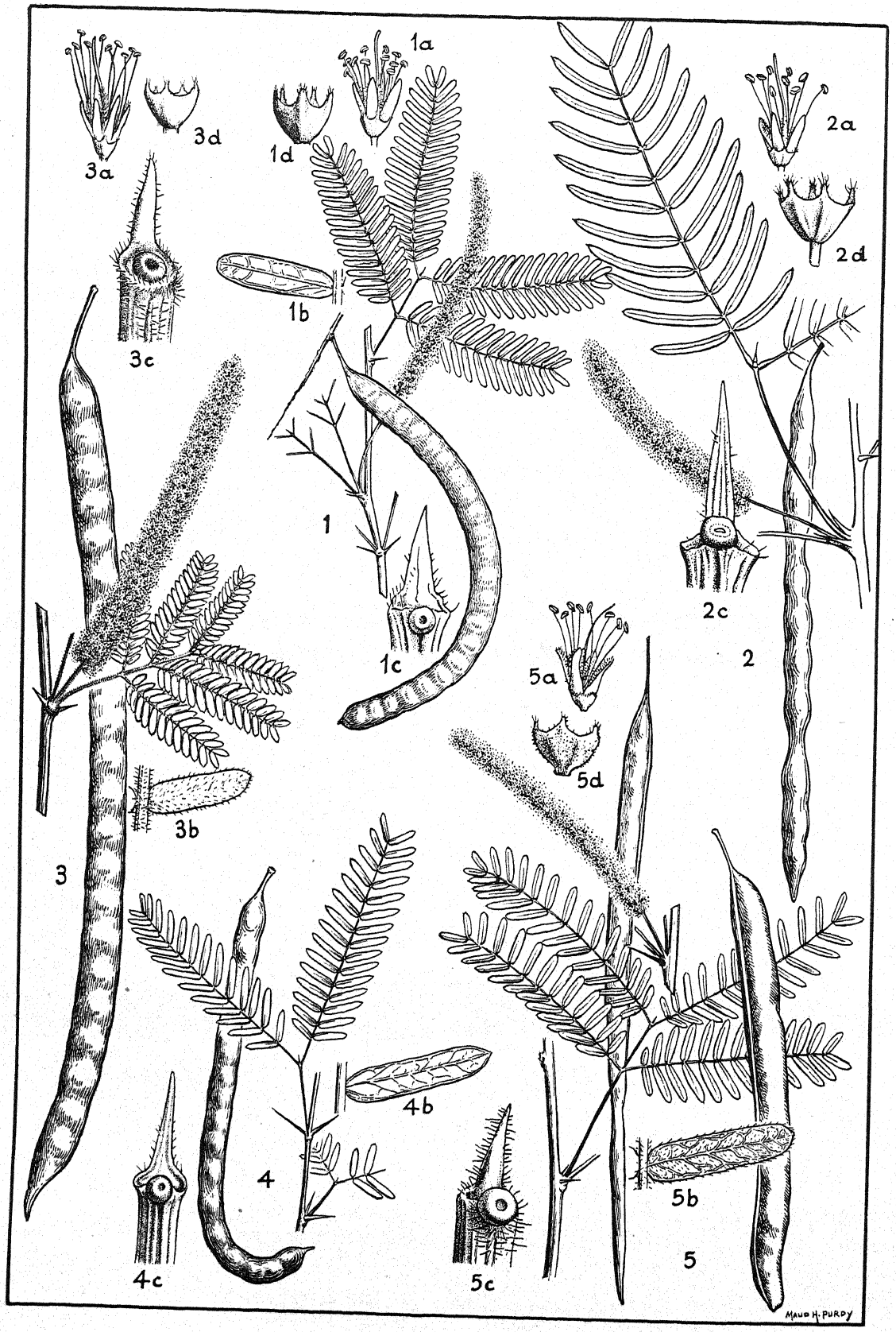
Small spreading trees 8 ft. high on rocky north slope of Puntilla, Salinas (11,089) (cf. pl. 1, f. 4). The rachis and the lower surface of leaflets are strigillose-pubescent. To *P. inermis*, described originally from Guayaquil, belongs the pubescent *Prosopis* characteristic of the coastal area of Ecuador. Sometimes the leaves are pubescent on only one surface or even glabrous. The characteristic strigillose pubescence will be found, if only slightly, on the rachis and about the apical glands (i.e., at the point of origin of the last pair of leaflets) (Pl. 11, fig. 1, 4, 5).

Pubescent plants grade imperceptibly into typically glabrous *P. juliflora*, although the extremes might readily be taken for distinct species. On the Ecuadorean coast the pubescent phase is predominant; in the Galapagos Islands the glabrous and pubescent specimens are about evenly divided; on the northern coast of South America and in the West Indies the glabrous form is predominant, so much so that Fawcett & Rendle (l.c.) describe the species as "glabrous." Specimens such as *Pittier* 9170 from Caracas and *Curran & Haman* 1231 from Guanta, Venezuela, show some pubescence on the leaves. When the pod is immature it is flat and straight to falcate, and the immature seeds appear as low rounded prominences in the middle. The surface is characterized by cordlike anastomosing veins which run lengthwise. In age, the pod becomes swollen and pulpy and outline of the seeds is no longer discernible. The following specimens represent the pubescent [*P. inermis*] phase: ECUADOR: vic. Duran, *Rose & Rose* 23,587 (US); Puná I., *Eggers* 14,022, noted as having white flowers and yellow fruit; Guayaquil, *Schimpff* 219 (B); between Machala and Puerto Bolivar (*Hitchcock*); (GALAPAGOS ISLS., Wreck Bay, Chatham I., *Stewart* 1614; Gardner I., *Snodgrass & Heller* 623; San Cristobal I., coastal region near Puerto Chico, *Schimpff* 165 (B); Duncan I., *Howell* 9808; and many other collections). PERU: east of Talara, *Horton* 11,587.

Bentham, in Hooker's Jour. Bot. 4: 350. 1842, mentions the large-leaved glabrous *P. dulcis* var. *β domingensis* (DC.) Benth. as having been collected at Guayaquil by Hall, and that *P. inermis* is "probably a thornless form of *P. dulcis β*." Typical glabrous *P. juliflora* occurs frequently on the Galapagos Islands. It is represented by my collection (no. 176) from Charles Island, and by *Snodgrass & Heller* 852 from Abingdon Island.

The leaflets in *P. juliflora* are consistently of 10–16 pairs, quite different from the situation described in the Mexican *P. dulcis* Kunth, with 18–25 pairs. The name *P. dulcis* is perhaps untenable since the older name, *Acacia laevigata* Willd., is cited as a synonym. That, however, is said to have come from South America. *P. horrida* Kunth, Mimos. 106, t. 33. 1822, from "radices Andium orientalium et in arenosis prope littus Oceani Pacifici," with white flowers and an edible torulose legume, is perhaps a spinescent phase of *P. inermis*, though the localities cited are only in part applicable. Closely allied, though distinct, is *P. limensis* Benth. in Hooker's Jour. Bot. 4: 350. 1842, with very small leaflets in 6–15 pairs (pl. 11, f. 3). It extends from the Peruvian coast at Piura southward to Lima. Of this species Burkart (Darwiniana 4: 115. 1940) cites three specimens in the Gray Herbarium: Lima, *Cuming* 974 (co-type); Payta, *J. Ball* 4/13/1882; Rio Apurimac, 1000 m. alt., *Weberbauer* 5366 & 5901. It is also represented by *Haught* 198 from "12 miles east of Cape Pariñas"; in this collection the mature pods remain flattened and are not swollen at maturity.

In his excellent treatment of *Proposis*, Burkart (l.c., p. 114) in discussing *P. articulata* Wats. (*P. juliflora* var. *velutina* (Wooton) Sargent) states that this species does not belong with *P. juliflora* but with the group of *P. limensis* Benth. and *P.*


1a
1d
1b
1
1c
2a
2d
2c
2
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5a
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4b
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algarobilla Gris., with elongate pods resembling those of *Parkinsonia aculeata*. He specifically limits *P. juliflora* to the West Indies and northern South America. I had independently come to the same conclusion, that *P. glandulosa* Torr. (Pl. 11, f. 2) and *P. velutina* are entirely distinct from *P. juliflora*. It is hard to see why they should ever have been united under the same species (cf. in this respect Benson's recent treatment in Amer. Jour. Bot. 28: 749–753. 1941), but perhaps Sargent's incorrect illustrations of "P. juliflora" in Sylva N. Am. t. 106 and 107. 1892 (which are excellent portrayals of *P. glandulosa* Torr.) have had much to do with the situation.

The algarrobas are important trees in the desert regions of western South America, for firewood and for feeding stock. Their use has been most interestingly described by Bowman, Desert Trails of the Atacama, Amer. Geog. Soc., Special Pub. No. 5, 1924. Seeman (in Hooker's Jour. Bot. & Kew Miscel. 4: 241. 1852), in contemplating plants which could perhaps withstand salt water and be suitable for forestation of Ascension Island, says, "I only know two trees of this nature, the Overal (*Varronia rotundifolia* [*Cordia lutea*] and the Algarrobo (*Prosopis horrida*, Kunth); these are found in Ecuador and New Granada on the very verge of the ocean, and also in the most arid places of the Peruvian deserts, where sometimes for years nothing save dew is known to fall. They are moreover highly useful. The berries of the Overal form excellent food for poultry, and the Algarrobo produces a bean which is almost the sole support of the numerous horses, mules, donkeys, and goats of the arid regions." *P. chilensis* (Molina) Stuntz, a species with numerous leaflets, occurs chiefly in Chile and Argentina. It has been well discussed by Burkart (l.c.).

156. RHYNCHOSIA MINIMA (L.) DC. Prod. 2: 385. 1825; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 73, fig. 22. 1920. *Dolichos minimus* L. Sp. Pl. 726. 1753. Trailing over rocks, the yellow flowers only in bud on April 6, Punta Centinela (11,398). This is the plant with large thin rhombic leaflets (2–4 cm. diam.) identical with my no. 89 from Indefatigable I., Galapagos. The species is wide-ranging, from South Carolina into the tropics.

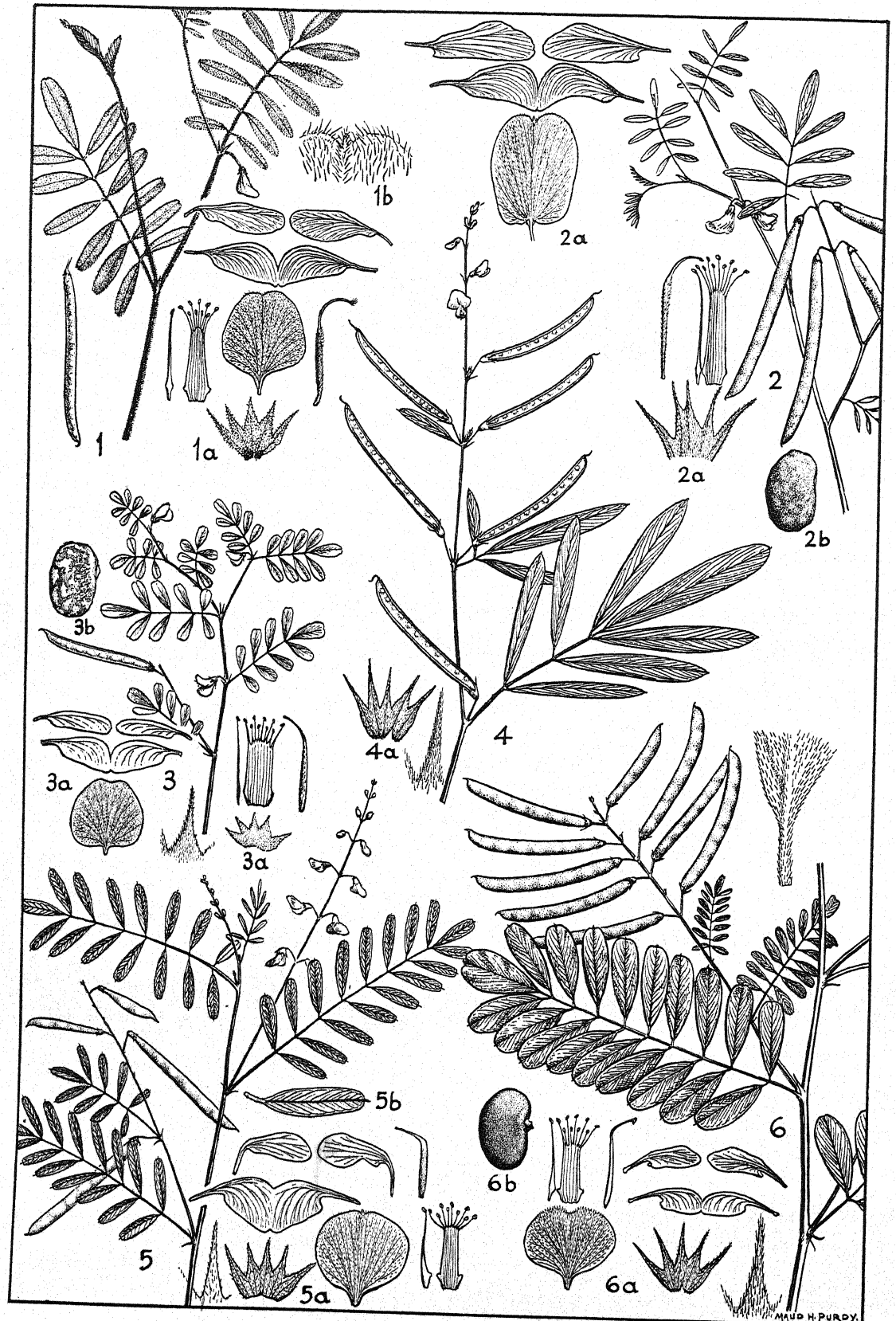
157. STYLOSANTHES SYMPODIALIS Taubert, Verh. Bot. Ver. Brand. 32: 19. 1891. *S. scabra* sensu Stewart (p. 78). Abundant in dry fields and sandy ridges at Salinas (11,400); Punta Centinela (11,258); hills south of Punta Carnera (11,301); and quite evidently the same as *Spruce* 2249 from Chanduy. Probably due to heavy rains of two seasons, plants which I collected frequently had larger leaflets (reaching 24 × 6 mm.) than Spruce's, which is well matched by *Howell* 8815 and my own collection (151) from the Galapagos Islands. Macbride (Fl. Peru, pt. 3: 412. 1943) cites *S. sympodialis* from the Amotape Hills (*Haught* F 44 and 134).

The Galapagos plant which Taubert saw at Vienna was characterized as "var. caulibus villoso-pubescentibus non scabris." All material which I have examined from the Galapagos Islands is *S. sympodialis* of the adjacent coast of Ecuador, with pubescence silky-white and somewhat spreading, becoming more abundant in the upper part of the plant. In *S. scabra*, described from Brazil and represented by *Burchell* 8533A and *Gardner* 973 from Pernambuco, the pubescence is brown, viscid, and incurving. The difference in pods is especially well marked (cf. Pl. 20, fig. 1 & 2), those of *S. scabra* being shorter (upper segment 3.5–4 mm. long, including the beak, and the lower segment 3 mm. long when developed), and the pubescence spreading-hispid and dark brown. In *S. sympodialis* the upper segment is 4–4.5 mm. long with the beak-apex curved to form a semi-circle. The lower segment is usually 3 mm. long. The strongly-reticulate surface has silky-white pubescence of varying amount, the pod being sometimes almost glabrous (cf. Pl. 20, fig. 1f).

The sympodial inflorescence of *S. sympodialis*, by which it is supposedly differentiated from all other species of the genus, has been described in detail by Taubert, but overtopping of the upper inflorescences by the lower is not constant. A diagram of the bracts is shown in figure 1c (from no. 151), showing the long pedicels after removal of the primary bracts and the corolla and calyx. The relationship in a single flower is shown to the left. In addition to the tubular bract which encloses the lower part of the calyx, two small bracts lie above the secondary bract and the "stipus plumosus." According to Taubert the "stipus plumosus" is not an aborted flower, but the terminus of a secondary axis, the same situation as in *Vicia monanthos* where the 1-flowered spike is a reduced raceme. The "stipus plumosus," characteristic of the section *Styposanthes*, is very prominent in *S. sympodialis*. The pubescence is more appressed than in *S. scabra*, the bracts are narrower and lack the purple midrib of *S. scabra*. In the Galapagos Islands there is variation in the amount of pubescence, but all specimens examined, such as *Howell* 8815 (Charles I.), *Snodgrass & Heller* 171 and *Stewart* 1695 (Albemarle I.) and *Baur* 89 (Jervis I.) belong to *S. sympodialis*. So probably does Andersson's plant from Puná Island, listed by Areschoug as *S. elatior*, a species of eastern United States.

158. TAMARINDUS INDICA L. Sp. Pl. 34. 1753; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 119. 1920. The tamarind, native to Africa and grown extensively

Plate 11. *Prosopis* (algarroba): branch and pod × ½; flower (a) and leaflet (b) × 2; gland terminating leaf-axis (c) × 7.5; calyx (d) × 7.5.—Fig. 1. *P. juliflora;* flowering branch (*Curran & Haman* 469, Venezuela); pod (*Pittier* 10,683, Venezuela).—Fig. 2. *P. glandulosa* (*Heller* 1465, Texas).—Fig. 3. *P. limensis* (*Haught* 198, Peru).—Fig. 4. *P. juliflora* (*Svenson* 176, Galapagos Islands).—Fig. 5. *P. juliflora* (*P. inermis*); flowering branch (*Eggers* 14,022, Ecuador); sterile branch and pods (11,089, Ecuador).


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6b
MAUD H. PURDY.

in tropical regions for its pulpy fruit, is semi-wild in places (Salinas, 11,282).

159. TEPHROSIA LITTORALIS (Jacq.) Pers. Syn. 2: 329. 1807. *Vicia littoralis* Jacq., Sel. Stirp. Amer. 206, t. 124. 1763. *T. cinerea* var. *littoralis* Benth. in Mart., Fl. Bras. 15, pt. 1: 48. 1859; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 21. 1920.

A low plant with dull bluish flowers, locally abundant in dry fields, Salinas (11,386); Punta Centinela (11,257); near La Brea, Piura, Peru (H&S 11,647). In this widespread species the pubescence is variable, also size of leaflets. To correlate both mainland and Galapagos material, representative figures have been drawn (Pl. 12). An average specimen of *T. cinerea* from the Bahamas (fig. 2) agrees well with the Galapagos plant (fig. 3), except that Galapagos specimens have dwarfed inflorescence, shorter calyx, and shorter pods (2.5–3.5 cm. long, and 6–8 seeded). Typical *T. cinerea* (of Fawcett & Rendle) has the "pod generally 8–10-seeded, 4–5 cm. long with minute white appressed hairs." In general Galapagos plants have appressed pubescence, but Robinson has noted (p. 155) a velvety spreading pubescence on stem or pedicels. *Vicia littoralis,* described by Jacquin from Cartagena, Colombia, has tomentose pubescence, and large abundant leaflets (to 7 pairs). Ecuadorean plants (fig. 1) closely resemble Jacquin's figure (t. 124) and specimens from his type locality, but collections from Pariñas Valley of Peru (identical with Haught's specimens from the same locality) have appressed pubescence, making them appear glabrous at first sight. Otherwise they are characteristic *T. littoralis.* Analogous variation in pubescence is found in the oriental *T. purpurea* (fig. 6), frequently confused with *T. cinerea* and related species in the New World (cf. B. L. Robinson, Bot. Gaz. 28: 193–202, 1899). It is evident from flower structure, as well as the heavy leaflets and pods, that *T. purpurea* is distinct from *T. leptostachya* (fig. 5) of southern United States, illustrated by *A. H. Curtiss* 561 and 584 from Florida (the type collection of *Cracca Curtissii* Small). Separation of species in *Tephrosia* by emphasizing appressed or spreading pubescence has been overdone. The identity of *T. caribaea* and *T. cinerea* in the West Indies is discussed by Schlechtendal in Linnaea 5: 180–181. 1830.

160. ZORNIA DIPHYLLA (L.) Persoon, Syn. 2: 318. 1807; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 31, f. 9. 1920. *Hedysarum diphyllum* L. Sp. Pl. 747. 1753. Annual with small yellow flowers hidden by prominent bracts; dry fields, Salinas (11,384). Widely distributed in the tropics of both hemispheres, and known from Albemarle Island in the Galapagos.

OXALIDACEAE

161. OXALIS CORNELLI Anderss., Eugenies Resa Bot. 1855. (Type from Galapagos Islands.) *O. Agassizi* Rose, Contrib. U.S. Nat. Herb. 1: 136. 1892. (Type from Galapagos Islands.) *O. ramulosa* Knuth, Notizbl. Bot. Gard. Berl. 7: 290. 1919. (Type from Piura, Peru, *Weberbauer* 5956.) *O. chanduyensis* Knuth, l.c. (Type from Ecuador, *Spruce* 6405.) *O. sepium* St. Hil. var. *glandulifera* Norlind, Ark. f. Bot. 20A, no. 4: 9. 1926. (Type from Puná I., Ecuador, *Eggers* 14,795.) *O. Barrelieri* sensu Knuth, Pflanzenr. IV 130: 65. 1930 (in part); Svenson, Amer. Jour. Bot. 22: 234. 1930.

A species with small orange-yellow flowers and pendulous pods, abundant on headlands of the Ecuadorean coast, Puntilla, Salinas (11,228). (Pl. 13, fig. 1 & 2). Often it is a slender annual with fibrous roots, as in no. 11,100 from open woods north of Santa Elena; or it may become a robust annual, as on beach at Punta Centinela (11,405); or a perennial with gnarled rootstocks, as on exposed headlands of the Puntilla, Salinas (11,077). Specimens collected at Quebrada Ancha, near La Brea, Peru, in gravelly soil (H&S 11,590) are robust annuals.

Most plants have a glabrous appearance, but young shoots always show a definite pubescence, generally of acicular hairs, but sometimes with glandular hairs interspersed. On lower parts of the stem, the hairs tend to elongate and collapse as the plant grows older, and these collapsed hairs sometimes have a glandular appearance. Size of leaves is variable: in robust material, as 11,405, leaflets reach 3.5 × 3.0 cm., but average length of the obtuse to emarginate leaflets is about 1.5 cm. *O. Barellieri* is a purple-flowered species, quite different from *O. Cornelli.* To add to the confusion, specimens from Piura (*Pennell* 14,816) have been placed by Knuth under *O. peruviana* (Standley) Knuth, which is quite a different plant. In addition to the specimens of *O. Cornelli* cited, I have seen *Hitchcock* 20,160 from Milagro, Ecuador; and *Horton* 11,551 and *Haught* 46 from Talara, Peru.

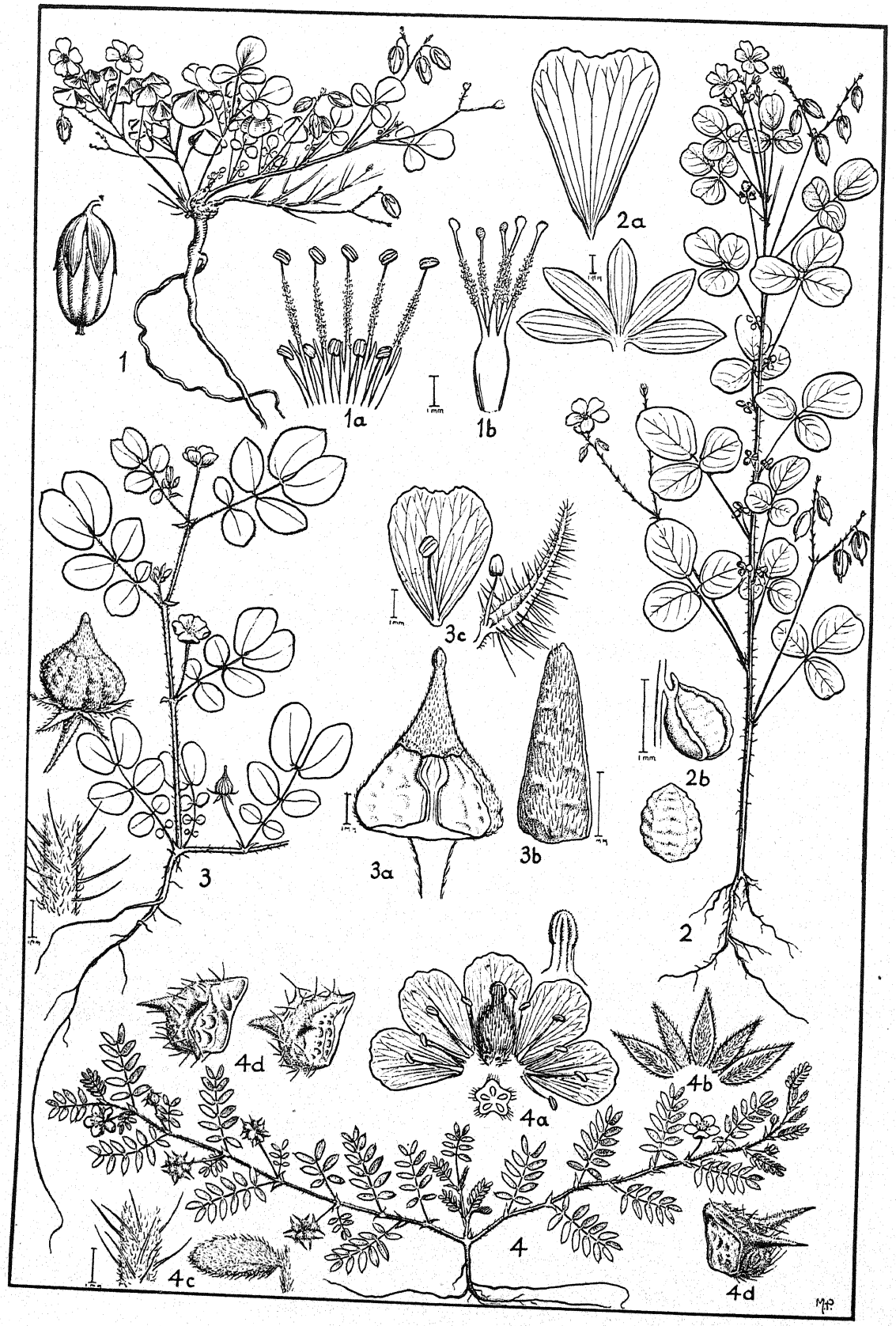
O. corniculata L., not uncommon on the Galapagos Islands, is characterized by slender erect capsules. Schimpff (no. 208) got it on Charles Island, and it is also represented by *Stewart* 1714, 1715, 1716, and 1718 from Charles and Chatham Islands, and by *Baur* 38 from Charles Island.

ZYGOPHYLLACEAE

162. KALLSTROEMIA ADSCENDENS Andersson, Eugenies Resa Bot. 245. 1855; Robinson, Proc. Amer. Acad. 38: 156. 1902. *K. tucumanensis* Descole, O'Donnell & Lourteig, Lilloa 4: 218. 1939, and 5: 266, t. & fig. 2. 1940.

This species (Pl. 13, fig. 3) is one of the earliest

Plate 12. *Tephrosia.*—Fig. 1. *T. littoralis* (11,386, Ecuador); dissected flower and calyx (1a); apex of leaf (1b).—Fig. 2. *T. cinerea* (*N. Taylor* 492, Bahama Is.) (2a), seed (2b).—Fig. 3. *T. cinerea* (*Howell* 9596, Galapagos Islands); dissected flower (3a); seed (3b).—Fig. 4. *T. littoralis* (11,647, Peru); calyx with a lobe enlarged (4a).—Fig. 5. *T. leptostachya* (*Curtiss* 5561, Florida); dissected flower (5a); leaflet × 1 (5b).—Fig. 6. *T. purpurea* (*Canton Christian College no. 8327,* China); dissected flower (6a); seed (6b).


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to flower after the spring rains. Prostrate plants along paths on escarpment north of Santa Elena (11,106) and on roadsides at Salinas (11,334). The inconspicuous white flowers have petals only 5 mm. long. From the excellent photograph and description in Lilloa, vol. 5, there can be no doubt that these Ecuadorean plants are the same as *K. tucumanensis* of western Argentina, though the latter is described as having pale yellow flowers. My Ecuadorean collections have large leaflets, some reaching even 23 × 13 mm., whereas the size given for *K. tucumanensis* is 7–13 × 5–9 mm., which is about the size of the leaflets in *Snodgrass & Heller* 615, from Gardner Island in the Galapagos, and the latter collection has leaflets indistinguishable from those of *Haught* 147 from Piura, Peru. In *Haught* 51, from the same region, leaflets have a maximum size of 17 × 9 mm. In all these collections the bluntness of the terminal segments is specially noticeable. Dr. Robinson (l.c.) thought that the Galapagos material was exceedingly close to *K. brevistylis* Vail, Bull. Torr. Club 24: 206. 1894, from southwestern United States. Macbride, Field Mus. Bot. 8: 119. 1930, has identified the Haught collections as *K. caribaea* Rydb., N. Amer. Fl. 25: 111. 1910, but in so doing has merely treated them as "this variant of the typical form of *K. maxima* (L.) Wight & Arnott, distinguishable only by its strigose fruit." These South American plants seem to be well distinguished from *K. terrestris* in the reduced number of leaflets and their relative broadness, weak stems, small flowers, and strigose fruits. *K. adscendens* is known from western Argentina, northwestern Peru, the coast of Ecuador, and the Galapagos Islands.

163. TRIBULUS TERRESTRIS L. var. SERICEUS Andersson, Eugenies Resa Bot. 225. 1855, and 107. 1861. *T. cistoides* var. *galapagensis* Svenson, Am. Jour. Bot. 22: 235. 1935. This is a common weed in sandy roadways in Salinas (11,087) and was also collected in dry fields near La Brea, Piura, Peru (H&S 11,568) (Pl. 13, fig. 4). It has the sericeous pubescence associated with the Galapagos plant, hitherto considered endemic to the Islands. Like the Galapagos individuals, it varies from densely hirsute to sparsely strigose-pubescent. At Salinas the plant begins to flower toward the end of February, and size of the plants depends on the immediate fertility of the soil. Some in pure sand flower when less than 2 cm. high (erect and unbranched), but in general they branch and spread out over the ground.

The most luxuriant plant, from a waste heap at Salinas (11,201), is identical with *Chapin* 1144 from Conway Bay, Indefatigable Island, except for shorter petals (8.5 mm. long as compared with 11 mm. in Chapin's specimen). In other plants (fresh random samples) the petals are shorter, as follows:

Length of petals in mm.	7.0	6.5	6.0	5.5	5.0	4.5	4.0
Number of flowers.....	9	26	35	37	44	25	13

The smaller-flowered Ecuadorean and Peruvian material (cf. Pl. 13, fig. 4) is densely strigose-pubescent both in leaf and fruit, just as in *Amdursky* 255 from Tel-Aviv, Palestine, and a collection by *Bonati* (in 1904) from southern France, the latter with petals 4.5–5.0 mm. long, and stamens 1 mm. long. The Ecuadorean plants, with stamens varying from 1.0–1.5 mm. long in the same flower, closely resemble Hutchinson & Dalziell's illustration of *T. terrestris* in Fl. Tropical W. Afr. 1: fig. 53. 1927. In respect to pubescence, it may be mentioned that *T. lanuginosus*, described originally from Ceylon by Linnaeus, has been generally accepted as a synonym of *T. terrestris*. Engler (Nat. Pflanzenf. 19a: 176. 1931) says it is probably only a variety of *T. terrestris* with larger flowers and thick woolly leaves. *T. terrestris* has the smallest flowers of any species. The carpels bear 2 or 4 large spines and numerous elevations with interspersed small spines. In this polymorphic plant, numerous species have been based on size of fruit, number of spines, on light or heavy pubescence, not only in North Africa and Nubia, but also in South Africa. Oliver has considered *T. cistoides*, distinguished by more erect growth and larger leaves, flowers and fruit, as a variety of *T. terrestris*. Schinz has followed his opinion and treated *T. Zeyheri* also as a variety of *T. terrestris*. Parallelling *T. cistoides* var. *anacanthus* Robinson of the Galapagos Islands in the absence of spines are *T. terrestris* var. *inermis* Boiss. Fl. Orient. 1: 902. 1867, from Kordofan, and *T. Pechuilii* O. Kuntze, from southwest Africa. *Tribulus* has its great development in the Old World, and since both *T. cistoides* and *T. terrestris* are generally looked upon as migrants into the New World, a solution of actual relationships of the Ecuadorean plants must await a clear knowledge of Old World species. Furthermore, the Galapagos plant is probably identical with *T. alacranensis* Millsp., Field Mus. Bot. 2: 54. 1900, from Pajaros Island, Yucatan.

RUTACEAE

164. MONNIERA TRIFOLIA L. Sp. Pl. ed. 2, 986. 1862. A small herbaceous plant with trifoliate leaves and small white flowers hidden in green bracts; plantation openings, Manglaralto (11,436). Widespread in tropical South America, described originally from Loefling's collection from Cumaná, Venezuela.

Plate 13.—Fig. 1. *Oxalis Cornelli;* perennial phase (11,077, Ecuador), with capsule enlarged, stamens and pistils (1a, 1b) of 11,228.—Fig. 2. *O. Cornelli;* erect annual phase (11,228, Ecuador) with enlarged petal and calyx (2a); seed (2b) free and within seed-covering.—Fig. 3. *Kallstroemia adscendens* (11, 106, Ecuador), with immature fruit and a fragment of stem; mature fruit (3a) with some carpels removed; a single carpel (3b) dorsal view; petal and sepal (3c).—Fig. 4. *Tribulus terrestris* (11,087, Ecuador); dissected flower (4a), with detail of style; calyx (4b); lower surface of leaf and fragment of stem (4c); 3 mature carpels showing variation in sculpture (4d).

BURSERACEAE

165. BURSERA GRAVEOLENS (HBK) Triana & Planch., Ann. Sci. Nat. ser. 5, 14: 303. 1872; Engler in DC. Mon. 4: 49. 1883. *Elaphrium graveolens* HBK., Nov. Gen. & Sp. 7: 31. 1824. *Bursera malacophylla* Rob., Proc. Am. Acad. 38: 160. 1902. Abundant small trees at head of inlet, Punta Ayangue (11,364 & 11,338); rock slides near base of Cerro Prieto, Amotape Hills, Piura, Peru (H&S 11,630). This is the fragrant Palo Santo of the Galapagos Islands, where the pubescent-leaved *B. malacophylla* represents only a trivial form, which is practically duplicated in no. 11,338. The leaves in both areas vary from acute to nearly obtuse. The type of *B. graveolens* came from Guyaquil (see fig. 13).

POLYGALACEAE

166. MONNINA CHANDUYENSIS Chodat, Bull. Herb. Boiss. 2: 167. 1893. Resembling a tall *Polygala* with flattened strongly-veined fruit. Occasional in sandy fields at Salinas (11,207) and the Puntilla, Salinas (11,221); and abundant on rocks at Punta Centinela. On rocky slopes of Cerro Prieto, 1000 ft. Piura, Peru (H&S 11,567). The species was described from Chanduy (*Spruce* 6398).

167. POLYGALA ALOPECURUS Chodat, Mon. Polyg. [Mem. Soc. Phys. Hist. Nat. Geneve 31], pt. 2: 227, t. 24, fig. 6–7. 1893. Forming large patches in sandy fields at Salinas (11,206 & 11,399) and southeast of La Libertad (11,192). According to Mr. Hunter this "purple-flowered grass" appears only in occasional years. This is apparently the first collection since the original plants were obtained by Spruce (no. 3397) from Chanduy. It is very close to *P. leptocaulis* Torr. & Gray, Fl. N. Am. 1: 130. 1838, and to *P. paludosa* St. Hil., Fl. Bras. Merid. 2: 8. 1829 (under which *P. leptocaulis* is included by Blake in Contrib. Gray Herb. n.s. no. 47: 97. 1916). In Chodat's key (p. 223) the aril of *P. alopecurus* is said to be ½ as long as the seed, but this is evidently a mistake since the illustration shows an aril only 1/10 the length of the seed, which does not differ in any respect from the seed of *P. paludosa* in Chodat's figure 5a. The seeds of *P. alopecurus* average 1.1 mm. long; those of *P. paludosa* are stated by Blake to be 1.2 mm. long, and *Pennell & Killip* 5963 from El Valle, Colombia (determined by Blake as *P. leptocaulis*), has seeds 1.5 mm. long, of texture and pubescence just as in *P. alopecurus*. It is questionable whether *P. alopecurus* represents more than a small-seeded variation of the widespread *P. leptocaulis*, which extends from Mississippi and Texas into northern South America.

EUPHORBIACEAE

168. ACALYPHA SUBCASTRATA Areschoug, Eugenies Resa Bot. 3: 137. 1910; Pax in Engler, Pflanzenr. IV 147. xvii: 42. 1924. Annual 1–2 ft. high, in underbrush, Chanduy Hills, 1200 ft. (11,043); thickets, Quebrado Mogollon, Amotape Hills, Peru (H&S 11,511). It was originally collected by Andersson on Puná Island, but I have seen it from the following collections (US): ECUADOR: Milagro, *Hitchcock* 20,212; Bahia de Caraquez, *Mille* in April, 1938; Balao, *Eggers* 14,251. PERU: Choza, Tumbez, *Weberbauer* 7699 (as *A. setosa* Richard). The species is close to *A. polystachya* from which it differs in being slightly pilose, also in the shorter staminate spikes (not exceeding 6 mm. in no. 11,511), and in the much shorter involucral lobes. It is even closer to *A. ostryaefolia*, which has staminate spikes and involucral laciniae of similar texture, even as to stalked glands. The seeds of *A. subcastrata* are practically smooth and only 1.5 mm. long, in these respects differing markedly from the above-mentioned species. According to Pax (l.c.) *A. polystachya* ranges from Mexico to Guatemala, with an old record (Pavon) from Peru; *A. ostryaefolia* grows from New Jersey to Mexico.

169. A. VILLOSA Jacq., Stirp. Amer. 254, t. 183, fig. 61. 1763. Herb 2–3 ft. tall with cordate leaves and minute green flowers, Chanduy Hills, 1000 ft. (11,050). A widely-distributed species, described by Jacquin from Carthagena, Colombia.

170. CROTON LOBATUS L. Sp. Pl. 1005. 1753; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 5: 483. 1924. A variable annual with three-parted leaves, in thickets and on dry rocks, Puntilla, Salinas (11,123); Punta Centinela (11,392); La Libertad (11,294); Chanduy Hills, 500 ft. (11,015); near La Brea, Piura, Peru (H&S 11,632). The species, originally from Vera Cruz, Mexico, is widespread in the American tropics.

171. C. RIVINAEFOLIUS HBK., Nov. Gen. & Sp. 2: 80. 1817. Type from Guayaquil.

C. alnifolius HBK. l.c. (Type from Guayaquil); not Lamarck.

C. quitensis Spreng., Syst. 3: 872. 1826. Based on *C. alnifolius* HBK.

"C. xalapensis Humb.?" Hook. f. Trans. Linn. Soc. 20: 188. 1847. (Type from James I., Galapagos); not *C. xalapensis* HBK.

C. brevifolius Anderss., Eugenies Resa Bot. 241. 1855. Type from Charles I., Galapagos.

C. Pavonis Muell.-Arg. Linnaea 34: 115. 1865. Type from Peru.

C. Jamesoni Muell.-Arg. in DC. Prod. 15, pt. 2: 549. 1866. Type from Guayaquil.

C. alnifolius var. *quitensis* Muell.-Arg. (op. cit., p. 604). Based on *C. alnifolius* HBK.

C. Scouleri Hook. f. var. *grandifolius* Muell.-Arg. (op. cit., p. 605). Based on *C. xalapensis* Hook. f.

C. Scouleri Hook. f. var. *brevifolius* Muell.-Arg. (op. cit., p. 605). Based on *C. brevifolius* Anderss.

C. Scouleri Hook. f. var. *castellanus* Svenson, Am. Jour. Bot. 22: 239. 1935. Type from Tower I., Galapagos.

Croton bushes are very abundant on the ridges near Ancon and Santa Elena, but I saw no trace of them on the Puntilla at Salinas or in the sandy country to the east of Salinas or south of Ancon, or in the Talara-Amotape region of northwestern Peru. These bushes are the same as on the Gala-

pagos Islands, except that forms with greatly reduced leaves do not seem to occur on the mainland. The species is apparently always dioecious. The leaves of the type of *C. rivinaefolius* are, from description, 12–13 cm. long and 4–6 cm. wide. Such leaves are represented by 11,353 from Punta Ayangue and are identical with those of my collection (no. 72) from 200–600 ft. on Indefatigable Island (illustrated in Amer. Jour. Bot. 22: Pl. 4, fig. 5. 1935). Specimens collected on the beach at Manglaralto (such as 11,468) appear to be identical with a photograph of *C. Pavonis,* which was said to have come from Peru. From its similarity to Ecuadorean collections, I suspect that the original material came from Guayaquil, and was obtained by Pavon's student, Tafalla. It is the same as *C. rivinaefolius,* but with leaves a little smaller. Large-leaved forms are characteristic of moist sheltered areas: in no. 11,056 from the summit of the Chanduy Hills the leaves are 16 × 11 cm., and those on the beach at Manglaralto approach this size. On rocky banks exposed to dry south winds, as the south slope of the cerro at Santa Elena, *C. rivinaefolius* is reduced to smaller-leaved forms (11,380 and 11,370) indistinguishable from *C. Scouleri* var. *brevifolius* of the Galapagos Islands, as represented by *Chapin* 1126 from South Seymour and *Pool* 290 (Astor Expedition) from Daphne Island. This smaller form is *C. alnifolius* HBK., stated by Muell.-Arg. to have leaves 4–7 × 2–2.5 cm. Between these extremes I collected all sorts of intermediates: for example, specimens from Punta Ayangue (11,348, 11,354, and 11,355) which are similar to *Schimpff* 214 from Charles I.; from Santa Elena (11,138 & 11,144, with some leaves nearly orbicular); from dry soil between Ancon and Santa Elena (11,184); and from summit of Chanduy Hills (11,057). *Hitchcock* 20062 from an Oil Camp between Guayaquil and Salinas (issued as *C. salviaefolius* HBK., vel. aff.) has slightly dentate leaves up to 6 × 3 cm., and is the same as *Stewart* 1830 (*C. Scouleri* var. *brevifolius*) from James Island. Of *C. Jamesoni* I have seen only a photograph; it is a nearly leafless specimen collected on the "cerro of Santana, Guayaquil, Dec., 1845," a locality now within the city limits. On the somewhat humid sandy beach at Manglaralto, *Croton rivinaefolius* grew at its best, associated with species such as *Hippomane Mancinella, Scaevola Plumieri,* and *Cyperus ligulatus,* of which I saw no sign on the coast southward. For the most important varieties of the Galapagos Islands I am recombining the following names:

C. RIVINAEFOLIUS var. **Scouleri** (Hook. f.) n. comb. C. Scouleri Hook. f. Trans. Linn. Soc. 20: 188. 1847; *C. Scouleri* var. *genuinus* Muell.-Arg. in DC. Prod. 15, pt. 2: 605. 1866.

C. RIVINAEFOLIUS var. **Macraei** (Hook. f.) n. comb. *C. Macraei* Hook. f. Trans. Linn. Soc. 20: 188. 1847.

C. RIVINAEFOLIUS var. **albescens** (Anderss.) n. comb. *C. albescens* Anderss., Eugenies Resa Bot. 242. 1855.

Specimens from various zones of humidity on the mainland afford a comparison with Galapagos collections; no. 11,468 from Manglaralto is close in appearance to *Stewart* 1836 (300–1300 ft.) at Villamil, Albemarle I.; *Stewart* 1840 (1000–1200 ft.) at James Bay, James I.; and *Stewart* 1835 (100–1650 ft.) from Abingdon I. No. 11,370, the reduced form from Santa Elena, and the similar no. 11,299 from hills north of Punta Carnera, are the equivalents of *Snodgrass & Heller* from 2000 ft. on Narborough I., *Snodgrass & Heller* from Wenman I., *Stewart* 1830 (to 1000 ft.) from James I., and *Stewart* 1811 and 1828 from Academy Bay (to 300 ft.), Indefatigable I.

Bentham (Bot. Sulph., p. 165) referred *Hartweg* 695 from Guayaquil to *C. rivinaefolius,* stating that he had staminate flowers only, whereas Kunth described a pistillate plant. Areschoug (p. 147) noted that specimens obtained by Andersson on Puná Island conformed well with *C. rivinaefolius* in the Willdenow herbarium. The gland at the leaf-base is an evanescent structure frequently entirely lacking, and best developed in the large-leaved forms. *C. rivinaefolius* is close to *C. Ruizianus* Muell.-Arg. of the Lima region (cf. especially *Worth & Morrison* 15,692, from Chala, Dept. Arequipa, 350 m.), but differs in the less pubescent capsules, less rugose leaves and smaller seeds. It is also undoubtedly close to the monoecious plants represented by *Rusby* 70 (B) and 51 (B) (as *C. urucurana* Baill.) from the vicinity of Canamina, Bolivia, and to the *C. flavens-C. discolor* group of the Caribbean region.

We may now imagine a *Croton* of the *brevifolius-grandifolius* type establishing itself on the Galapagos Islands from the adjacent dry coast of Ecuador, where it occurs abundantly. Where it penetrates into the comparatively moist areas of the Galapagos it produces the "grandifolius" phase; where it reaches into the most barren parts of the islands it deteriorates into the very small-leaved and albescent phases which seem to be unknown on the Ecuadorean coast. In this respect the behavior is the same as that of *Waltheria ovata* and *Abutilon umbellatum,* to mention only two outstanding examples. It should be noted that *Croton rivinaefolius* is not associated with the xerophytic flora of the Piura-Chanduy-Puntilla region, but with a more inland zone which parallels the coast in a northwest-southeast direction, and which reaches the sea in the neighborhood of Punta Ayangue. One of the most interesting problems associated with the Galapagos Islands, therefore, is why microphyllous forms should have been developed on the Galapagos Islands and not on the mainland. The climatic conditions on the dry mainland and on the Islands are imperfectly known, and the variations from season to season in both places are so unpredictable that available records might mean but little. However, the prevailing dry southwesterly winds which blow in on the coast of Ecuador and Peru from

across the Humboldt Current are probably much harder on vegetation—and this would be especially true of fruticose types—than are the winds or lack of them on the southern shores of the individual Galapagos Islands. The size of shrubby vegetation in the arid parts of these islands—let us say Tower Island—is approximately what one finds on the Santa Elena and Punta Ayangue escarpments of the Ecuadorean coast. The Peruvian area at Talara, both north and south, is much more desert-like than anything that I have seen in the Galapagos Islands, and this applies also, though in a lessened degree, to the lowlands extending from Chanduy to Salinas on the Ecuadorean coast. Another interesting phase of the Galapagos *Croton* is the transition from the *grandifolius* to the lanceolate-leaved and finally the *Macraei* type on the shore (Academy Bay) of Indefatigable Island (cf. Amer. Jour. Bot. 22: chart on p. 211, and Pl. 4, fig. 5, 2a, and 4a). It is significant that here the bushlike forms seen on the mainland and on Tower Island (l.c., Pl. 8, fig. 4) actually become slender trees (Pl. 9, fig. 1) some 15 feet in height. With this reduction of leaf-surface the silvery-lepidote character which is pronounced in the var. *albescens* begins to make its appearance; in neither the pistillate nor staminate inflorescence of these narrow-leaved forms at Academy Bay is there any alteration from that of the broad-leaved plant of the Ecuadorean mainland. This area back of Academy Bay has in general been considered to be the most humid region of the Islands; and it is interesting to find here a series of leaf forms apparently not duplicated on the drier sides of the islands. Reduction in the *Macraei* type proceeds much further on some islands, such as at Tagus Cove on Albemarle, to the point where the leaves become cylindrical or even thread-shaped.

172. DALECHAMPIA SCANDENS L. Sp. Pl. 1054. 1753; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 5: 492. 1924. Pubescent vine climbing over shrubs, Manglaralto (11,456, in fruit). Widely distributed from the West Indies to tropical South America.

173. EUPHORBIA GRAMINEA Jacq., Stirp. Amer. 151. 1753. Erect plants with white involucral glands, plantation clearings, Manglaralto (11,485). In this collection with narrowly lanceolate leaves, the seeds are 1.5–2 broad and 1 mm. long, terete with acute apex, gray with minute reddish dots, and with broad quadrate surface indentations. The species was described from Carthagena, Colombia.

174. E. aff. GRAMINEA Jacq. Stem and leaves pubescent, plantation clearings, Manglaralto (11,486). In this collection the leaves are much broader (to 5 × 2 cm.). The seeds (2 × 1.5 mm.) are covered by wart-like excrescences.

175. E. aff. GRAMINEA Jacq. Glabrous, stem woody below, leaves somewhat verticillate, rocky slopes of Cerro Prieto, Piura, Peru (H&S 11,620). Seeds 2.5 × 2 mm., ashy gray, becoming black, the surface with irregular obtuse ridges.

176. E. HIRTA L. Sp. Pl. 454. 1753. Fawcett & Rendle, Fl. Jam. 4, pt. 2: 337. 1920; Wheeler, Contrib. Gray Herb. 127: 68. 1939. *E. pilulifera* L. Sp. Pl. 454. 1753. *E. capitata* Lam. Encyc. 2: 422. 1788. Along railroad, Salinas (11,335). An abundant prostrate tropical weed with yellow capsules and flowers in dense, mostly sessile heads; the typical form as treated by Wheeler. *E. hirta* was described in Fl. Zeylanica (p. 88), but *E. pilulifera* seems never to have had a Linnaean description. From this point of view the species was based primarily on the plant in Hermann's herbarium, which was the basis of Fl. Zeylanica. The two Linnaean species were first united by Lamarck under the new name, *E. capitata,* with the remarks, "We do not find it convenient to observe all the slight differences that the very variable plants of this genus offer and to multiply the species to infinity, as Linnaeus and other botanists frequently do; therefore we unite here the two plants, which from our observation on both living and herbarium specimens, we are forced to regard as varieties of one species." *E. pilulifera* was therefore placed under *E. capitata* β. Lamarck not only introduced the unnecessary name, *E. capitata,* but included under *E. hirta* many elements—references to Plumier, Sloane, and Rumphius, which never formed any part of the Linnaean name—and omitted any reference to Flora Zeylanica. After this he wrote *"Euphorbia hirta.* Lin. La mal nommée [there badly named]." This is not precisely the situation as it is referred to by Wheeler (l.c.): *"E. capitata* Lam. Encyc. Meth. Bot. 2: 422. 1786, substituted for *E. hirta* on the ground that the name was bad," for Lamarck supplied new names indiscriminately. *E. hirta* is an abundant weed in both hemispheres, and is known from the Galapagos Islands.

177. E. HIRTA L. var. DESTITUTA Wheeler, Contrib. Gray Herb. 127: 70. t. 4, fig. 1. 1939. An erect plant with narrow leaves on dunes and sandy flats, Salinas (11,071, 11,157, & 11,333); dunes, Punta Centinela (11,270). This plant has been previously known only from Piura, northwestern Peru (colls. *Weberbauer* and *Haught*). The internodes are long and nearly glabrous. The cyathium is much less developed than in typical material, and when glands are present they frequently have a whitened border. The hairs below the inflorescence are characterized by orange banding at the junction of the cells, as in typical *E. hirta.* Seeds average 0.7 × 0.3 mm.

178. E. LASIOCARPA Klotszch, Nov. Act. Caes. Leopold Nat. Cur. 19, Suppl. 1: 414. 1843. Abundant on exposed headlands and sea cliffs, especially within reach of salt spray, Salinas (11,079, 11,083, 11,234–11,236, & 11,319); banks of ravine, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,519); rocky slopes, Cerro Prieto, Piura, Peru (H&S 11,604). It was also collected by Haught (no. 199) at Caña Dulce, 35 mi. east of Cabo Blanco, as an upright herb (Nov. 13, 1927), with notation that he had not seen it elsewhere.

A large number of collections were made by me to show the transition from pubescent plants with

hairy capsules to plants perfectly glabrous throughout (nos. 11,234 and 11,083). They vary from erect to prostrate, from obvious annuals to perennials with thick roots, and with involucral glands ranging from white to dark pink. These variations of a single species occur indiscriminately in plants growing side by side. The seeds are usually elongate (averaging 1.1 ×0.45 mm.), with a rather flat dorsal surface frequently white-coated when mature, and usually with transverse ridges. *E. lasiocarpa* was collected from the vicinity of Lima; a collection made by Andersson on San Lorenzo Island near Callao (US) agrees very well with the original description, and is the same as my Ecuadorean collections. The glabrous plants have a striking resemblance to *E. flabellaris* of the Galapagos Islands, and the relationship is probably close. But *E. flabellaris* has shorter seeds which are foveolate, and I think it is a distinct species. Specimens from the Caribbean area have been treated as *E. lasiocarpa,* but the seed-dimensions (1.1 × 0.7 mm.) given by Millspaugh, Field Mus. Bot. 2: 397. 1914, and the figure in Bot. Gaz. 25: 17. 1898, differ radically from the Peruvian-Ecuadorean plants. *E. lasiocarpa* has a strong resemblance to the North American *E. Preslii,* but has larger seeds and more prominent glands.

179. E. MICROMERA Boiss. ex Engelm., Proc. Amer. Acad. Arts & Sci. 5: 171. 1861; Wheeler, Contrib. Gray Herb. 136: 194, pl. 658C. 1941. A prostrate small-leaved annual, near La Brea, Piura, Peru (H&S 11, 586). Mexico and southwest United States; collected by Pennell (no. 14,815) at Paita (cf. Wheeler).

180. E. THYMIFOLIA L. Sp. Pl. 454. 1753; Fawcett & Rendle, Fl. Jam. 4, pt. 2: 342. 1920. Prostrate reddish plants along railroad, Salinas (11,287). An abundant tropical weed of both hemispheres. The leaves are not as blunt as in most material examined.

181. PHYLLANTHUS aff. ORBICULATUS L.C. Rich., Act. Soc. Nat. Hist. Paris 1: 113. 1792; Muell.-Arg. in DC. Prod. 15, pt. 2: 401. 1866. Slender annual with orbicular leaves, shaded rocks on slope of Cerro Prieto, 1300 ft., Piura, Peru (H&S 11,631). *P. orbiculatus* is widely distributed in the American tropics.

ANACARDIACEAE

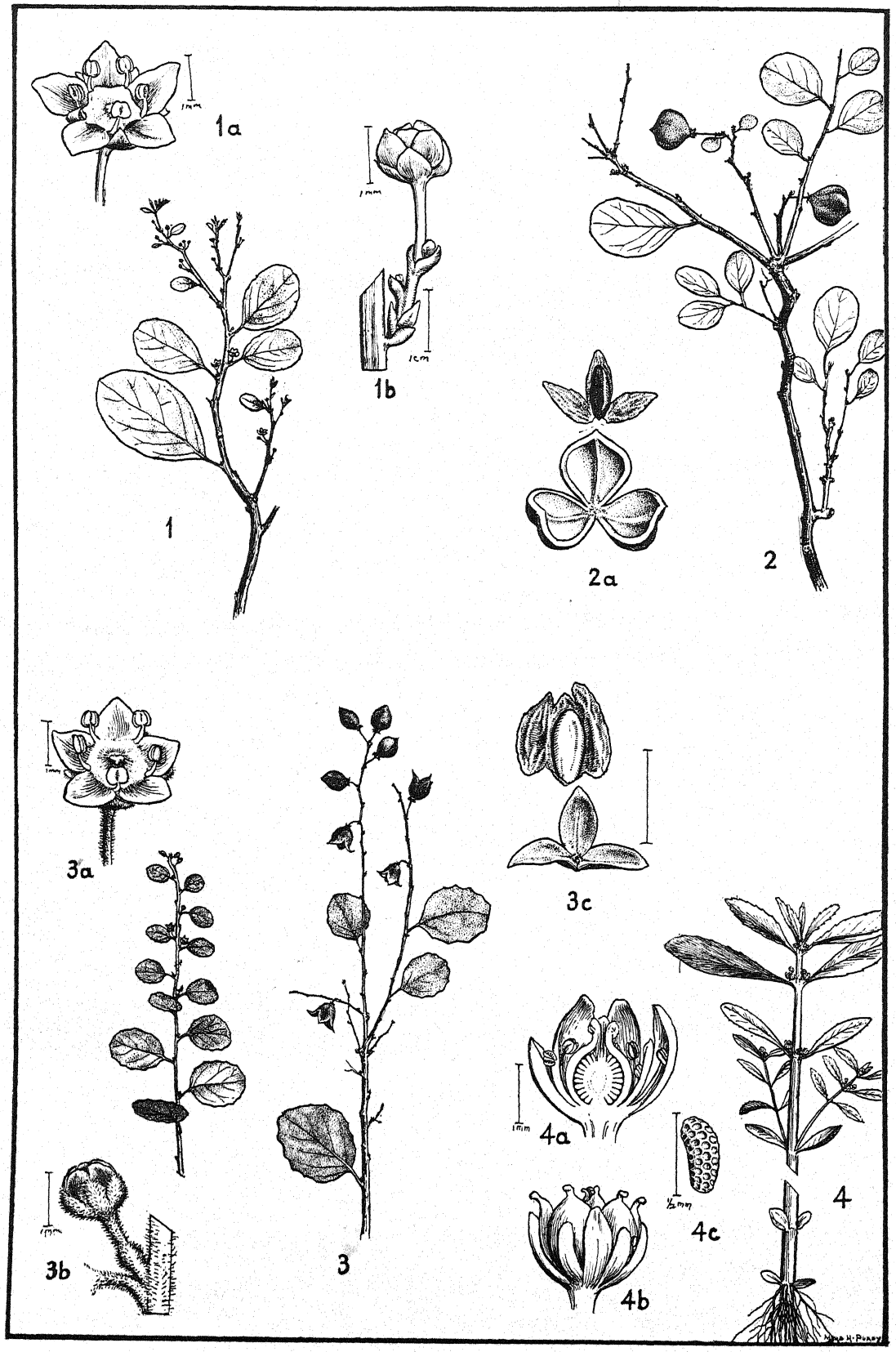
182. LOXOPTERYGIUM HUASANGO Spruce ex Engler in DC. Mon. 4: 459. 1883; Weberbauer in Engler & Drude, Veg. der Erde 12: 96, fig. 13. 1911. This is an abundant rounded tree, often 30 ft. tall, on the ridges near Santa Elena (11,375), and it forms extensive groves on the rolling land between Santa Elena and Ancon. It is not found on the sandy area west of Santa Elena or on the Puntilla. The bark and leaves have a resin which affects the skin much as our related poison-ivy (*Rhus Toxicodendron*), but this is the only tree in the area with timber value and it is therefore used extensively for house frames and for fence posts. Sometimes it puts forth green leaves before the rainy season starts. This dioecious tree occurs also in the grassy country back of Chanduy (where it was originally collected by Spruce). It is of scattered occurrence in the foothills of northwestern Peru, and is one of the three trees mentioned by Weberbauer (l.c., p. 153) as characteristic of the desert region. Quebrada Ancha, near La Brea, Piura (H&S 11,558) (Pl. 3, fig. 5, 6; pl. 5, fig. 7).

CELASTRACEAE

183. MAYTENUS OCTOGONA (L'Hér.) DC. Prod. 2: 9. 1825; Svenson, Amer. Jour. Bot. 22: 241. 1935. *M. obovata* Hook. f. Trans. Linn. Soc. 20: 230. 1847. *M. orbicularis* (Willd.) Loesener in Engler, Bot. Jahrb. 50, Beibl. 111: 10. 1913.

Sprawling shrubs on the inner dunes at Salinas (11,310) (becoming erect and often 10 ft. high farther inland), Santa Elena (11,168) and Chanduy Hills, 1600 ft. (11,042). Abundant also in northwestern Peru: near La Brea (H&S 11,573). The fleshy leaves vary from ovate to suborbicular or obovate, with margins often prominently repand-dentate. The fruit is an ovate bluntly-trigonous capsule, usually 3-celled, but sometimes 2- or 4-celled. My own Galapagos collection with entire leaves and thick-walled capsules (Pl. 14, fig. 2) appeared so different from continental material that at one time I considered it as distinct. But specimens from the islands show all sorts of transition from glabrous to pubescent twigs or calyx, and from thin-walled to thick-walled capsules, and from dentate to entire leaves. In some cases the disc is a little more distinct than in others, as shown in the illustrations (Pl. 14, fig. 1–3). Plants like *Baur* 48½ from Chatham Island have orbicular, dentate leaves and glabrous twigs; others, like *Stewart* 1925, have obovate leaves, pubescent twigs, and rather thick-walled capsules; some, like *Baur* 49 from Albemarle Island, have obovate leaves, thin capsules and practically glabrous twigs. In the Galapagos Islands the species is found at sea level on most islands, and extends to about 1000 ft. on Charles and Chatham Islands, and 650 ft. on Duncan I. (*Von Hagen* 76A & 76B). An old specimen of *Celastrus octogonus* in the Gray Herbarium (from the Boston Society of Natural History), perhaps representing authentic Peruvian material, is practically glabrous. A specimen with "minutely pubescent young branches" was mentioned by Riley, p. 220, from James Island.

Flowers are borne singly on pedicels 1–2.5 mm. long. The pedicels, which are frequently bracteate, occur in twos or threes at a single leaf-axil. *M. obovata* is close in appearance to *M. phyllanthoides* Benth. of the Caribbean-Mexican region and to *M. Vitis-idaea* of Argentina, but differs especially in the broad, long-pedicelled fruits. In shape of the disc and petals it is very close to *M. phyllanthoides*. *M. octogona* was based on a *Dombey* collection from Peru. *M. uliginosa* came from the vicinity of Guayaquil, and *M. orbicularis* was evidently based on the


1a
1mm
1
1b
1mm
1cm
2
2a
3a
1mm
3
3b
1mm
3c
4
4a
1mm
4b
4c
½mm

same collection; by Reissek (cf. Loesener in Engler, Bot. Jahrb. 36: 381. 1905) the two were considered synonymous, and by Loesener and Kunth they were considered at least as close species. I have not seen the original of L'Héritier's description.

Additional continental specimens which I have seen are from: CHILE: Tacna, *Rusby* 2618 (NY). PERU: Talara, *Haught* 58 & 76; Paita, *Haught;* Rio de Lomas, 600–700 m., *Weberbauer* 5740 (US); western slopes of the Andes above Pisco (Dept. Ica), 1000–1200 m., *Weberbauer* 5381 (US). ECUADOR: Playas (Prov. Guayas) *Mille* 183; Oil Camp between Salinas and Guayaquil, Hitchcock 19,991; El Recreo, Manabi, *Eggers* 1547; Bahia de Caraquez, *Mixter* 1296 (US). Loesener (1913), in discussing the two mountain collections of Weberbauer cited above, states that the leaves are narrower and thicker than usual, and the identity is not certain. They grew along a stream in rocky soil sparsely covered by plants. To me they appear identical with the glabrous specimens with obovate leaves from the Galapagos Islands.

SAPINDACEAE

184. CARDIOSPERMUM CORINDUM L. Sp. Pl. ed. 2, 526. 1762; Radlkofer, Pflanzenr. IV. 165: 397. 1932. *C. galapageium* Rob. & Greenm. Proc. Am. Acad. 32: 38. 1896. Vine with woody base and inflated pods, ridge north of Santa Elena (11,369); Chanduy Hills (11,053); Quebrada Mogollon, Amotape Hills, Peru (H&S 11,525). All these belong to f. *villosum* (Miller) Radlk. in Mart. Fl. Bras. 13, pt. 3: 447. 1897, though no. 11,525 has almost entirely glabrous pods. Under f. *villosum* belongs *C. galapageium,* a variation with unusually narrow leaves. This variable species is widely distributed in the tropics of both hemispheres.

RHAMNACEAE

185. SCUTIA SPICATA (Willd.) Weberbauer, Field Mus. Bot. 8: 83. 1930; Svenson, Amer. Jour. Bot. 22: 242. 1935. Rounded spiny bushes, forming thickets just above the tidal mark, Salinas (11,148) (Pl. 1, fig. 2); abundant shrub, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,540). The stout green thorns, often 6 cm. long, make passage through thickets of these shrubs impossible. The small red fleshy fruits are sour but very palatable. This shrub is abundant in the Galapagos Islands. A recent collection by *Ynes Mexia* (no. 4076) extends it to 1800 m. altitude, Chosica to Matucana, Dept. Lima, Peru.

VITACEAE

186. CISSUS SICYOIDES L. Syst. ed. 10. 897. 1759; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 5: 539. 1924. Vine climbing over bushes, Manglaralto (11,441); dwarfed plants on rocks and dunes, Puntilla, Salinas (11,226 & 11,284), and Punta Centinela (11,401); glabrous vines climbing over bushes, Chanduy Hills (11,006). This variable and widespread vine is represented in two distinct forms. No. 11,441 has tomentose branchlets and lower leaf surface, thin petals 1.5 mm. long and rotund anthers only 0.5 mm. long. It perhaps represents f. *compressicaulis* (R.&P.) Planchon in DC. Mon. 5: 531. 1887, described, in part, from Guayaquil. The other collections are wholly glabrous, with thickened petals 2 mm. long, and elongate anthers 1 mm. long. Possibly two distinct species are represented. *C. sicyoides* is known from Florida and Mexico southward to Paraguay, and on the Galapagos Islands.

ELAEOCARPACEAE

187. MUNTINGIA CALABURA L. Sp. Pl. 509. 1753; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 89, fig. 39. 1926. Trees 1 ft. diam. with white flowers and red fruit, Ancon (11,187). These trees may have escaped from cultivation, since the fruit, known as "frutilla," is eaten as a sauce. It is about the only fruit, except the tamarind, which will grow in this dry climate.

TILIACEAE

188. CORCHORUS ORINOCENSIS HBK. Nov. Gen. & Sp. 5: 337. 1823; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 86, fig. 38. 1926. A yellow-flowered aquatic annual at borders of pools east of Salinas (11,414), described from Venezuela. Another collection (11,194, from pools east of La Libertad), has ovate leaves and is without fruit; but is probably only a broad-leaved form.

MALVACEAE

189. ABUTILON CRISPUM (L.) Medic. Malv. 29. 1787; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 98, fig. 42. 1926. *Sida crispa* L. Sp. Pl. 685. 1753. Trailing annual with minute orange flowers and inflated capsules. On dry rocks, Puntilla, Salinas (11,248); Punta Centinela (11,397); Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,543). In these collections the carpels are single-seeded though the species generally has 3-seeded carpels. I can find no other distinctions. Described from Jamaica, it is widespread from southern United States to South America and in the Old World tropics, and is known from several of the Galapagos Islands.

190. A. UMBELLATUM (L.) Sweet, Hort. Brit. 1: 53. 1826; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 97. 1926. *Sida depauperata* Hook. f. Trans. Linn. Soc. 20: 232. 1847. *A. Anderssonianum* Garcke in

Plate 14.—Fig. 1. *Maytenus octogona;* flowering branch of glabrous form (*Von Hagen* 7, Galapagos Islands) with mature flower (1a) and unopened flower (1b) enlarged.—Fig. 2. *Maytenus octogona;* fruiting branch of glabrous form (*Svenson* 40, Galapagos Islands) with capsule, seed, and aril in detail (2a).—Fig. 3. *Maytenus octogona;* flowering and fruiting branch of pubescent form (11,168, Ecuador) with mature flower (3a), and unopened flower (3b), and capsule with seed and aril (3c).—Fig. 4. *Bergia capensis* (11,033, Ecuador); longitudinal section of flower (4a); calyx with expanded capsule walls (4b); seed (4c).


1b
1e
1c
2a
1a
1d
1f
1
2
3
4b
4
4a
5
6
7
7a
MAUD H PURDY

Andersson, Eugenies Resa Bot. 230. 1855, and 98, t. 15. 1861.

Yellow-flowered perennial about 2 ft. high with cordate leaves. Flowers borne in clusters at the top, or the lowermost often long-pedicelled and solitary in the leaf axils. Carpels 6–8. Dry soil of Puntilla, Salinas (11,253). This plant, not in flower until about April 7, and consequently one of the last species to bloom in the rainy season, is the phase with long bristly pubescence of the calyx, characteristic of the Ecuadorean coastal region, and represented by *Mille* 116 and *Mathews* (G) from Guayaquil, and by *Weberbauer* 5967 from between Chulucanas and Morropon, alt. 150 m., Piura, Peru.

A reconsideration of the status of *Abutilon* in the Galapagos Islands has not caused me to change my opinion (cf. Amer. Jour. Bot. 22: 243. 1935), and I still believe that the plants called *A. depauperatum* and *A. Andersoniianum* belong under *A. umbellatum*. Throughout the species, glandular hairs impart a viscid character to the pubescence, which tends to be more compact in Galapagos plants than in the continental specimens; it is sometimes very short and close in depauperate material, such as *Snodgrass & Heller* 76 (Pl. 15, fig. 2) from Iguana Cove, Albemarle Island, but the Galapagos plants lack, in general, the long simple hairs seen in continental specimens.

As in *Lantana, Croton,* and *Waltheria,* one finds great reduction in the foliage and the inflorescence. Hooker described Darwin's collection of *Sida depauperata* as having short 2–3-flowered pedicels, a rufous-ferrugineous calyx with obtuse segments, corolla ⅓ of an inch long, and 5–6 carpels with 3–5 seeds each. Fruiting specimens of the Galapagos plant which I have seen usually have 7 (sometimes 6) carpels, and the carpels rarely have, in my experience, more than 3 seeds. The illustrations by Andersson (t. 15) are not at all convincing, since in figure f, 12 carpels are shown, whereas the number is stated in the text to be 8 or 9. The carpels of *Abutilon* are strongly loculicidal, and the lateral half of each carpel remains attached to the corresponding half of the adjacent carpel even at maturity. What at first sight may seem to be a 2-awned carpel, therefore, represents the fusion of two halves of adjacent carpels, and I cannot help but feel that confusion of the sort has entered into the illustrations and description of *A. Anderssonianum.* Mature seeds are infrequent, but those of *Schimpff* 48 from Indefatigable Island and of *Broadway* 412 from Cristobal Colon, Venezuela, appear identical, 2 mm. long, of triangular outline, with a glutinous-brown surface, irregularly marked by sharp tubercles. Broadway's collection I cannot distinguish from *Snodgrass & Heller* 702 from Duncan Island. *A. umbellatum* ranges from Mexico and the West Indies to northern Colombia and Venezuela. It reappears in the Galapagos Islands and on the dry coast of Ecuador and Peru.

191. CIENFUEGOSIA HETEROPHYLLA (Vent.) Garcke, Bonplandia 8: 148. 1869; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 5: 566. 1924. *Redoutea heterophylla* Ventenat, Pl. Cels. 11, t. 11. 1800. Annual or perennial, the yellow flowers with petals 2–4 cm. long, reddish-brown at the base. Fields, Salinas (11,328); river gravels, La Brea, Piura, Peru (H&S 11,584). The leaves are variable, ranging from lanceolate or obovate to digitate or deeply tripartite. The dull white tomentum on the seeds, resembling that of cotton, is about 1 cm. long, much longer than the seeds themselves. These collections vary from the Ventenat description and plate chiefly in the thickened and pubescent style and in the broader calyx lobes. From Guayaquil, Bentham, Bot. Sulphur 68. 1844, described *Fugosia cuneata,* a procumbent plant with cuneate-bifid to entire leaves. Garcke (p. 149), from description, placed it close to *C. heterophylla,* of which I believe it is perhaps only a weak stage. *C. heterophylla* was described from St. Thomas, in the Virgin Islands, and ranges from Florida to Brazil and Peru, where it was collected by Haught from Cerro Prieto (no. 191), and from Talara (no. 40).

192. MALVASTRUM **depressum** (Benth.) n. comb. *Sida depressa* Bentham, Bot. Sulphur 69. 1844. *Malvastrum dimorphum* Howell, Proc. Calif. Acad. Sci. ser. 4, 21: 331. 1935. Shrubs 2–4 ft. high with orange-yellow flowers and densely-tomentose leaves, Puntilla, Salinas (11,247 & 11,251); hills north of Punta Carnera (11,300). On disintegrated granite, Quebrada Mogollon, Amotape Hills, Peru (ca. 400 ft., with *Capraria peruviana, Boerhaavia tuberosa,* and *Cryptocarpus pyriformis*), (H&S 11,521).

Sida depressa was obtained from nearby Puná Island and the description is so relatively short that I am including it here in translated form.—"SIDA *depressa,* sp. n., fruticose? viscid-tomentose, leaves cordate ovate, crenate, canescent below, the peduncles very short, calyx viscid-tomentose, carpels 10, pubescent, the exterior angles mucronate.—Isle of Puná, near Guayaquil. Habit similar to that of *Bastardia viscosa.* Tomentum short, viscid, canescent. Leaves 1–1½ inches long, the petiole ½ inch. Flowers the size of those of *S. rhombifolia.* Calyx after anthesis enlarged and stellate-spreading. Fruit

Plate 15.—Fig. 1. *Abutilon Anderssonianum* (*Snodgrass & Heller* 662, Galapagos Islands); details of flower (1a, 1b) × 1.5; fruit (side view with calyx removed) (1c), top view (1d), and longitudinal (1e) and horizontal cross-section (1f).—Fig. 2. A reduced form, probably *Abutilon depauperatum* (*Snodgrass & Heller* 76, Galapagos Islands), with 1-flowered inflorescence; fruit (2a) enclosed in calyx; and stellate hairs.—Fig. 3. *Abutilon umbellatum* (*Broadway* 412, Colombia), with fruit (enclosed in calyx); longitudinal section of capsule × 1.5; and a seed × 5.—Fig. 4. *Abutilon umbellatum* (11,253, Ecuador), an extreme with long pubescence on calyx and carpels; a single carpel (4a), side view × 2.5; corolla × 1; calyx (4b) with fruit × 1.5.—Fig. 5. *Malvastrum dimorphum* (*Schimpff* 35, Galapagos Islands) fruit and carpel × 1.—Fig. 6. *Malvastrum scabrum* (*Hitchcock* 20,043, Ecuador).—Fig. 7. *Sida depressa* (type coll.) flower (7a) × 1.

depressed, stellate-radiate, separating into distinct carpels which are very slightly dehiscent."

The type collection (Pl. 15, fig. 7) of which there is a specimen in the Gray Herbarium, has small leaves only, and flowers aggregated on short pedicels. These flowers are 1.5 cm. in diameter, and together with carpels and calyx, are exactly the same as *Schimpff* 35 (hb. Brooklyn) cited as representative of *M. dimorphum* (Pl. 15, fig. 5), which is known only from Indefatigable Island in the Galapagos. A collection by Hitchcock from between Salinas and Guayaquil (issued as *M. scabrum*) is also figured (Pl. 15, fig. 6). It seems to me that all three collections clearly represent the same species, since the flowers, calyx, and carpels are identical. *Mille* 162 from Guayaquil, undoubtedly the same species (issued as *M. scoparium*), has a strong resemblance to the photograph of Lamarck's type of *M. scoparia,* but the resemblance is perhaps only superficial.

As Howell has kindly pointed out to me, the calyx-lobes of my collections are broader and shorter (cf. Pl. 16, fig. 6), than in *M. dimorphum.* The calyx diameter in *Schimpff* no. 35 according to my measurements, is 20 mm.; in no. 11,247, 15–19 mm. The calyx-lobes in *Schimpff* 35 are 8–10 mm. long and 4 mm. wide; in no. 11,247 they are 5 × 4 mm., and of thicker texture. Leaves in my collections are more velvety, perhaps because the plants grew in exposed places. By some botanists they would be considered as a distinct variety, but Hitchcock's and Mille's specimens have a calyx of the same texture and size as the Schimpff collection. In describing *M. dimorphum,* Howell has taken into consideration the larger flowers and fruit than in *M. scoparium,* described by L'Héritier from Dombey's collections near Lima, and cultivated at Paris. Also to be taken into consideration is *M. scabra* (Cavanilles) A. Gray, Bot. U.S. Expl. Exped. 1: 147. 1854, from Huanuco Province in Peru, and likewise cultivated at Paris. Both of these Gray considered as a single species, and with them he included *Sida depressa: "*[*M. scabrum*] differs (perhaps too slightly) from *Malvastrum scoparium* . . . . in the more coarsely and sharply toothed leaves, which are not canescent, and in the shorter tubercles rather than awns on the back of the carpels, which moreover are muticous at the proper apex. *M. scoparium* (to which apparently belongs *Sida depressa,* Benth. Bot. Boy. Sulph. p. 69), a common plant around Lima, though not in the present collection, has a short and erect cusp at the strongly incurved apex of the carpels, as is described by Cavanilles."

The genus *Malvastrum* was united with *Sphaeralcea* by Kearney. Now it appears that *Malvastrum* cannot be clearly differentiated from *Sida.* The genus *Sida* L. was founded upon dissections of living material (cf. introd. Gen. Pl. § 24 (1737), and the illustrations of *Malvinda* Dill., Fl. Elthamensis t. 171 (*Sida spinosa,* f. 210; *S. cordifolia,* f. 209) and t. 172 (*S. rhombifolia,* f. 212; *S. alnifolia,* f. 211); together with the species of *Abutilon* Dill. t. 2 (*Sida cristata*), t. 5 (*S. crispa*) and t. 6 (*S. occidentalis*), in conformity with the references in Species Plantarum. By Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 5: 550. 1924, *S. alnifolia* has been taken as the type or standard species of the genus *Sida.* This species was described in Fl. Zeylanica, grown in European gardens from seeds originating in Bengal.

By Bentham & Hooker (Gen. Pl. 1: 197. 1862) the *Eumalveae* (including *Malvastrum*) are characterized by an ascending ovule, the *Sideae* by a pendulous ovule. It is not easy to follow this idea, but I believe it is connected with the relative length of the central axis to which the carpels are attached. Where the axis is very short the capsules tend to lie horizontally, as in *Anoda* and *Sida depressa;* where the axis is elongate, the capsules tend to stand upright and to be attached at their apex, as in *Sida spinosa.* The illustrations (Pl. 16, fig. 3a, 6) will make this clearer. However, Bentham by including *Sida depressa* within the genus *Sida,* showed that either the idea was not a rigid one, or had not come into being until the later date (1862) of the Genera Plantarum. Schumann (in Engler & Prantl III. 6: 32. 1895) noted that the seeds of *Malvaceae* are of two transitional types. Either they are attached at the inner angle of the carpel, and tend to stand upright, or they hang down from the upper part of the angle, whereby the raphe is bent outwardly. In *Abutilon* and in other genera, both types can be observed in the same capsule. As Asa Gray says of *Malvastrum* (Bot. U.S. Expl. Exped. 1: 146. 1854): "This genus is distinguished from *Malva* by the capitate stigmas; from *Sida* by the peritropous-ascending ovule and inferior radicle. The species with a manifest involucel have commonly been referred to the former, and those with a small or caducous involucel, or none at all, to the latter genus." Both purple-flowered and yellow-flowered species were included.

193. SIDA ACUTA Burm., Fl. Ind. 147. 1768; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 119. 1926. *S. carpinifolia* L. f. Suppl. 307. 1781. A weedy shrub 2–4 ft. high with orange flowers, Manglaralto (11,443). Abundant around dwellings from Florida southward, and in the Old World tropics. It is known from Charles Island in the Galapagos.

194. S. CAMPESTRIS Benth. Pl. Hartw. 113. 1843. *S. spinosa* sensu Rob., Proc. Am. Acad. 38: 175. 1902 (in part) and Stewart, Proc. Calif. Acad. Sci. ser. 4, 1: 103. 1911 (in part). *S. angustifolia* sensu Robinson (l.c. in large part). *?S. linifolia* Juss. ex Cav. Diss. 1: 14, t. 2, f. 1. 1785. *?S. salviaefolia* Presl, Rel. Haenk. 2: 110. 1836.

Usually dwarfed, often unbranched annual (Pl. 16, fig. 1), though sometimes reaching 1 m. in height, with narrow crenate-serrate leaves and small white flowers with red center, opening only in the morning. Dry rocky soil on the Puntilla, Salinas (11,249); fields and roadsides, Salinas (11,315); on granite, Quebrada Mogollon, Amotape Hills,

Peru (H&S 11,521A). Collected by Hitchcock from between Guayaquil and Salinas (no. 20,116) in a more robust form. In the Galapagos Islands it is known from Narborough I. (*Stewart* 2036); Tagus Cove, Albemarle I. (*Snodgrass & Heller* 206 (G)); Daphne I. (*Wheeler, Rose & Beebe* 8); Charles I. (*Svenson* 165). The plant perhaps occurs in Venezuela: vic. Maracaibo (*Pittier* 10,545 and 10,679); vic. Didivi, Truxillo (*Pittier* 10,864); sandy shores at Puerto Colombia (*Pennell* 12,026). But the flowers in Pennell's collection are described as "maize-yellow."

Plants from the Guayaquil region have 5–8 carpels, nearly black, with two retrorse barbs of variable length terminating the beaks. The lateral ribs of the carpels form spinose projections. I have not seen original material of *Sida campestris,* described from Guayaquil, but the description agrees in all respects with my own collections and that of Hitchcock, except in the number of carpels. Bentham's description says that the carpels are "subdenis" [i.e. sub-10]; in the discussion he says that *S. campestris* differs from *S. rhombifolia* and *S. angustifolia* in the rather long, subviscid stellate pubescence, with long simple hairs intermixed, and broad lower leaves; from *S. pilosa* in having 10 carpels, not 5.

There is some probability that the Ecuadorean plant may also represent *M. salviaefolia* Presl (*S. spinosa* var. *salviaefolia* Baker, Jour. Bot. 30: 237. 1892), described[13] from Acapulco, Mexico, as having carpels with retrorsely scabrous bristles, and as being allied to *S. angustifolia. Sida spinosa* (as may be seen from fig. 123 in Gray's Genera, and our illustration, Pl. 16, fig. 2) has much larger capsules (3.5–4 mm. long) with antrorsely-barbed bristles, though some reduced specimens from the Galapagos Islands (cf. *Schimpff* 46 from Indefatigable Island, Pl. 16, fig. 4) have capsules a scant 3 mm. long, and ovate to almost linear leaves 0.5–1.5 cm. long. The flowers are half the size of those of typical *S. spinosa;* 8 mm. as compared with 17 mm. in diameter. It may be mentioned that *Sida spinosa* on the Galapagos Islands shows the same signs of dwarfing that is seen in *Lantana, Waltheria, Croton,* and other genera. These dwarfed plants (with corresponding reduction in size of carpels) have fruit almost identical with Mexican plants such as *Stanford, Retherford & Northcraft* (carpels barely 3.5 mm. long) which have been identified as *Sida angustifolia* or *S. spinosa* var. *angustifolia* (Lam.) Griseb. Lamarck's original description of *S. angustifolia* was based on subshrubs 3 or 4 ft. high with yellow flowers, with almost linear leaves, grown at Paris from seeds originating in Mauritius or Reunion.

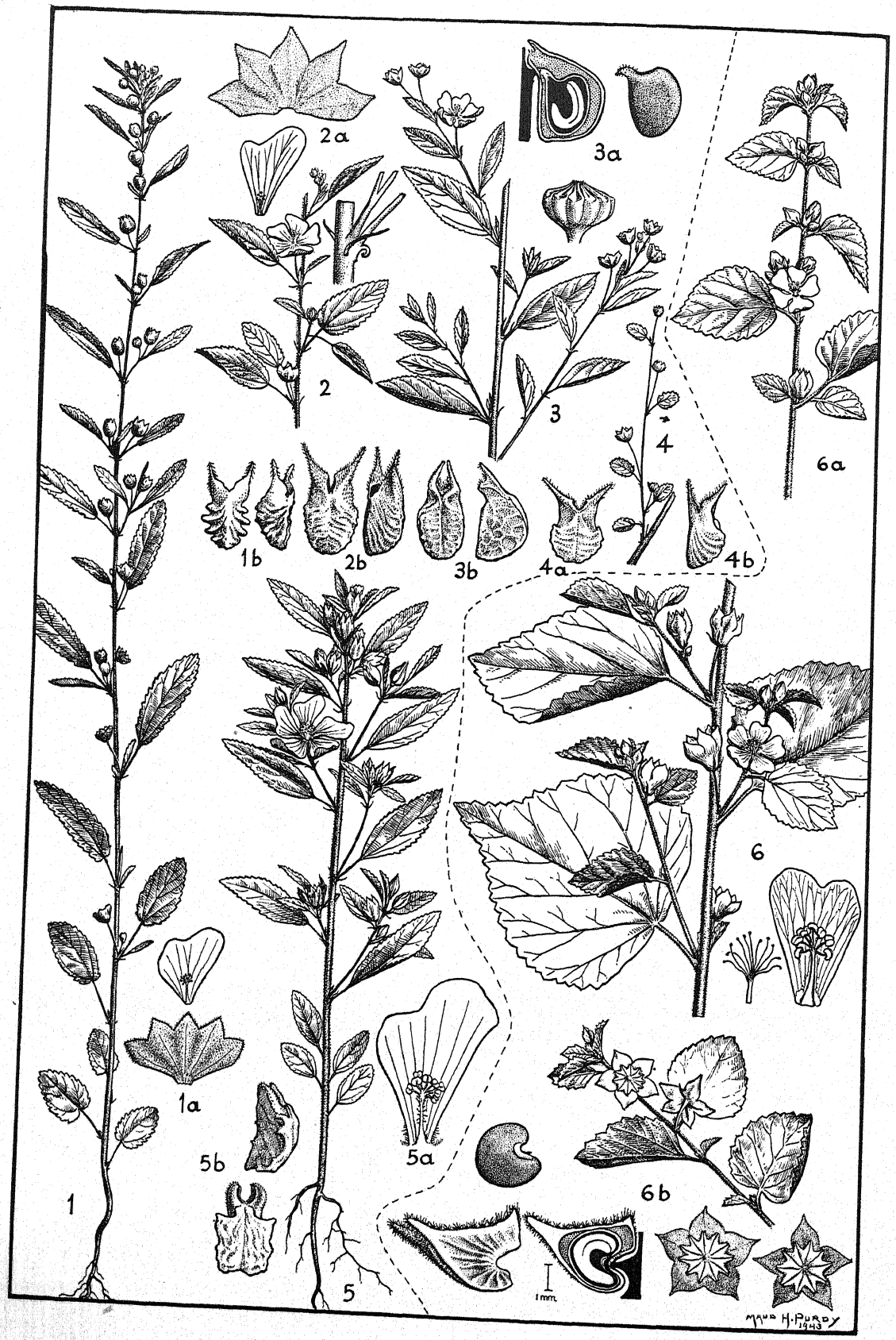
[13] *Sida salviaefolia* Presl. S. annula. Corollam non vidi. Carpella sex, longe biaristata, rugosissima, glabra, furca, dorso longitudinaliter profunde canaliculata, margine obtuse dentata, aristis loculo ipso aequilongis pilis rigidis retrorsis scabris. Affin *L. angustifolia* sed diversissima. Calyx tres lineas longus, tomentosus, decemnervius, laciniis acutis. Aristis retrorsum scabris. Acapulco.

The identity of *Sida tenuicaulis* Hook. f., Trans. Linn. Soc. 20: 232. 1847, has not been verified, but the plant collected by Darwin was described as having linear-oblong leaves, brown above, and canescent below, and the stem "erecto ramoso lignoso," characters which would tend to place the plant as the dwarfed form of *S. spinosa.* By Robinson, who followed Baker (Jour. Bot. 30: 237. 1892), the Galapagos plant was included under *Sida angustifolia* Lam. Andersson (1861, p. 98) remarked that Hooker had compared the Galapagos plant with *S. spinosa,* but on Garcke's authority it was closer to *S. angustifolia* Lam., from which it differed in the lack of a spine at the base of the petiole and the leaves more rounded at the apex, and that it was closer to *S. salviaefolia* Presl in the smaller carpels included in the calyx. It may be mentioned that dwarfing is also apparent in *S. rhombifolia* in the Galapagos Islands, as in *Stewart* 2031 from Charles Island. In this collection, although an extensive woody taproot is developed, the stems rise only 10 cm. above ground, and the carpels (only 7) have the apical bristles greatly reduced. *Schimpff* 197, also from Charles I., is similar. The specimens of *S. acuta* collected by Schimpff on Chatham I. (no. 114) also show dwarfing.

195. S. CILIARIS L. Syst. ed. 10, 1145. 1759 and Amoen. Acad. 5: 401. 1759; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 115. 1926. Weed forming mats on roadsides and dunes, Salinas (11,238, 11,291, & 11,327). The small red flowers are only 12–14 mm. in diameter. This species was described in Amoen. Acad. from Jamaica, as having oval retuse leaves, and carpels spinescent on the back. In these respects my collections agree with the original description, but the carpels are not "saepius 8," but rather 6; in respect to carpel spinescence they are identical with such Mexican collections as *Pringle* 4497 and *Mexia* 1155, both from Jalisco. In various forms, *S. ciliaris* occurs from Florida and Texas southward into Central and South America, but it is not known from the Galapagos Islands.

196. S. PANICULATA L. Syst. ed. 10, 1145. 1759; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 114. 1926. Bushes 3–5 ft. high with panicles of purple flowers. Sandy border of beach, Manglaralto (11,462); summit of Chanduy Hills, 1800 ft. (11,061). The species was originally described from Jamaica. It is widespread from Cuba to Paraguay, and known from moist moderately elevated places in the Galapagos Islands. It occurs also in tropical Africa.

197. S. WEBERBAUERI Ulbrich in Engler. Bot. Jahrb. 54, Beibl. 117: 68. 1916. Erect grayish annual with abundant pink to white flowers, in dry soil, east base of Puntilla, Salinas (11,230); rocky slopes at base of Cerro Prieto, Piura, Peru (H&S 11,582). The species (Pl. 16, fig. 5) has hitherto been known only from Piura, where it was collected by Weberbauer at Hacienda Nomala, at Serran, and at Chancro, stations 5°–5°30′ S. lat. and at 100–300 mm. altitude. The plant has a weedy aspect, despite the rather large flowers (2–2.5 cm.


1
1a
1b
2
2a
2b
3
3a
3b
4
4a
4b
5
5a
5b
6
6a
6b
1 mm.
Mary H. Purdy 1943

diam.). At Salinas, the flowers had distinct color forms, the most abundant being pale rose with dark pink veins (11,230a), and almost equally so a form with dark red center (11,230). Plants with pure white flowers were infrequent (11,230b). *S. Weberbaueri* belongs to section *Malvinda,* and, according to Ulbrich, is most closely related to *S. acuta* Burm.

STERCULIACEAE

198. AYENIA aff. MAGNA L. Syst. ed. 10, 1247. 1759; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 160. 1926. Shrub 6 ft. high, summit of Chanduy Hills, 1800 ft. (11,017). The capsules are sessile; otherwise the flower greatly resembles the illustration of the Mexican *A. rotundifolia* Hemsley, Biol. Cent.-Am. 1: 135. 1879 and 5: t. 11. 1888. The cordate, soft-pubescent leaves of 11,017 are 5–9 cm. long and 4–6 cm. wide; the axillary inflorescences are 2-flowered, each flower on a pedicel 1 cm. long. The anthers are 3-lobed. The corolla lobes are 3-toothed, the central tooth being much longer. The crownlike portion of the corolla (situated between the lobes and above the stamens and readily detached) is nearly 1 mm. long, deeply 2-cleft, with a long whip-like process, about 3 mm. long, originating from the cleft.

199. BUETTNERIA GLABRESCENS Benth., Bot. Sulphur 71. 1844 (Pl.18, fig. 1). This abundant bush in the Santa Elena-Ancon region is formed of a clump of semi-woody stems rising to 8 ft. in height. These bear alternate glabrous cordate leaves, with the numerous greenish umbellate flowers on filiform peduncles in the axils. The stems are prominently ridged, but only rarely do they show any trace of spines. Ridge north of Santa Elena (11,139); north slope of Puntilla, Salinas (11,126); Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,538). This species was also collected by Hitchcock between Salinas and Guayaquil (no. 19,954) and by Eggers at Puná Island (no. 14,784) and at El Recreo (no. 15,617). It is rather close in appearance to *B. Dielsii* Mildbraed in Diels, Fl. Ecuador 106. 1937, as represented by *Schimpff* 460 from Huigra. *B. parviflora* Benth., of which *Schimpff* 373 from Western Guayaquil seems to be a representative collection, is a much more pubescent plant with smaller, less dentate leaves, and the inflorescence mostly a terminal panicle. The plants described from Peru by Cavanilles are not yet clearly recognized, and *B. glabrescens* may perhaps represent one of Cavanilles' species.

200. GUAZUMA ULMIFOLIA Lam. Encyc. 3: 52. 1789; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 157, fig. 58. 1926. Small trees along stream, Manglaralto (11,463). The leaves are densely tomentose below (var. *tomentilla* K. Schum. in Mart. Fl. Bras. 12, pt. 3: 81. 1886), a form known from Peru, and from Brazil to Mexico.

201. WALTHERIA OVATA Cav. Diss. 6: 317, t. 171, fig. 1. 1788; Svenson, Amer. Jour. Bot. 22: 244, pl. 3, fig. 4. 1935. *W. reticulata* Hook. f. Trans. Linn. Soc. 20: 231. 1847. *W. sericea* Turcz., Bull. Soc. Nat. Mosc. 31, pt. 1: 214. 1858. An abundant shrub, either prostrate or forming erect bushes to 6 ft. Open headlands, often in salt spray, Puntilla, Salinas (11,227 & 11,318); Chanduy Hills, 1200 ft. (11,040); Quebrada Mogollon, Piura, Peru (H&S 11,515). This plant shows a variation in leaves comparable to *Croton rivinaefolia*—i.e., none of the very small-leaved forms of the Galapagos Islands appear on the mainland, though large-leaved forms are characteristic of both areas. No. 11,515 is practically identical with *Schimpff* 180, from the dry coastal region of Chatham Island. It has cordate velvety leaves up to 4 × 3.5 cm. As the leaves become progressively smaller in the islands, the pubescence tends to become shorter, giving the leaves a veiny appearance. Characteristic of *W. ovata* is the sulphur-yellow color of the leaves, especially the lower surface, when seen in light reflected at an angle.

Fortunately there has been little complication in synonomy of this species of the west coast of South America. *W. ovata* came from Peru; *W. sericea,* based on *Jameson* 518, from Guayaquil (described as having ovate subcordate leaves), is without much question the same. *W. ovata* differs from *W. americana,* otherwise, in having filaments not united to the top. The description of *W. reticulata* emphasized texture of leaves and not their shape, since they were described as cordate-ovate or even linear-oblong.

ELATINACEAE

202. BERGIA CAPENSIS L. Mant. 241. 1771. *B. aquatica* Roxb. Corom. 22, t. 142. 1798. *B. verticillata* Willd., Sp. Pl. 2: 771. 1799. A small aquatic with minute flowers in axillary glomerules, in pools south of Ancon (11,033). (Pl. 14, fig. 4). This plant is widespread from Indo-China westward to Egypt, but is unknown at the Cape of Good Hope. I can find no specimens or records of its occurrence in the New World. Linnaeus was working on *Erica* of South Africa when he wrote up *Bergia,* and I believe the misconception as to its geographical origin so arose (cf. letter no. 543 to Bergius, in

Plate 16. (In species of *Sida,* calyx, style and petals are × 2, habit × 1; capsules are shown from dorsal view (to left) and lateral view (to right) × 4.—Fig. 1. *S. campestris* (11,315, Ecuador); calyx and petal (1a); capsules (1b).—Fig. 2. *S. spinosa* (*Harper* in 1907, Georgia); calyx and petal (2a); capsules (2b). Stipules and spinous process are enlarged.—Fig. 3. *S. rhombifolia* (*Demaree* 13,466, Arkansas); group of capsules (with bracts removed), seed, and diagrammatic section of capsule showing the relation of seed and cotyledons to columella (3a); capsules (3b).—Fig. 4. *S. spinosa* (*Schimpff* 46, Galapagos Islands), dwarf form; capsules (4a, 4b).—Fig. 5. *S. Weberbaueri* (11,230, Ecuador); petal, with stamen column (5a); capsules (5b).—Fig. 6. *Malvastrum depressum* (11,300, Ecuador); petal, stamens and styles (× 2); (11,521, Ecuador) apex of shoot (6a); (11,247, Ecuador) showing various forms of calyx × 2, capsule × 4, and seed (6b). Capsule with lateral wall removed shows relation of seed (in vertical section) and cotyledons, to the columella (cf. fig. 3a).

Hulth, vol. 1, pt. 3, p. 152–3. 1909) [translated]: ". . . . I intend to have among my new genera a *Bergia* but am undecided between two genera as to which one would please you more. One is an *herba capensis* of the stature of *Ammannia floribus verticillatis* of which the fruit or capsule after dehiscing resembles a little star. It cannot possibly be placed under *Sedum* for it is simply 5-locular and not 5-capsulate. I have only a single specimen of it. The other is a tree from the East Indies which grows in unknown mountains."

VIOLACEAE

203. HYBANTHUS CIRCAEOIDES (HBK) Baillon, Bot. Méd. 2: 841. 1844. *Ionidium circaeoides* HBK., Nov. Gen. & Sp. 5: 379, t. 498. 1823. Perennial, with opposite leaves and minute purplish flowers, scattered in the underbrush, Punta Ayangue (11,336). This species, of localized distribution on the Ecuadorean coast, was obtained by Mille at Bahia in 1938. It came originally from "prope Guayaquil locis umbrosis, ad littora maris Pacifici," so that the collection from Punta Ayangue may be close to the HBK. locality.

TURNERACEAE

204. TURNERA PUMILEA L. Amoen. Acad. 5: 395. 1760; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 229. 1926; Macbride, Fl. Peru, pt. 4: 84. 1941. Pubescent annual to 3.5 dm. with small yellow to cream-colored flowers hidden in the leafy apex. Thickets, north slope of Puntilla, Salinas (11,231 & 11,241); low grounds, Salinas (11,285); rocky banks, Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,644). A species of dry areas from Cuba to Peru.

CARICACEAE

205. CARICA PANICULATA Spruce ex Solms-Laubach in Mart. Fl. Bras. 13, pt. 3: 177. 1889; Macbride, Fl. Peru, pt. 4: 139. 1941. *C. leptantha* Harms, Notizbl. Bot. Gart. Berlin 8: 95. 1922.

Shrubs 3–6 ft. high on dry hillsides, with bright pink flowers and large dissected leaves (Pl. 4, fig. 6). North of Ancon (11,170); ridge north of Santa Elena (11,136); banks of stream at west base of Chanduy Hills (11,028). The leaves of this glabrous, dioecious species vary greatly in shape, being primarily 3-lobed with the middle lobe deeply pinnatifid. The lobes and their divisions are obtuse to acuminate. The staminate flowers have short lobes (only 3–4 mm. long) with a long tubular portion below, and appear on a much-branched panicle of which the naked peduncular part is 1–1.5 dm. long. The pistillate flowers are much larger (1.5 cm. long) and lack the tubular portion which is found in the staminate flowers; they are borne in corymbs of 5–6 flowers on short peduncles only 1–1.5 cm. long. *C. paniculata* was based on a *Spruce* collection from Chanduy, but it ranges to an altitude of 1000 meters in Cajamarca, Peru. Several localities in the Peruvian lowlands of Tumbez and Piura are cited by Macbride, an area in which the Ecuadorean plant would be expected to appear. The species is one of the few plants with brilliant flowers, on the arid coast of Ecuador. These often appear before the leaves.

LOASACEAE

206. GRONOVIA SCANDENS L. Sp. Pl. 202. 1753; Macbride, Fl. Peru 13, pt. 4: 144. 1941. Scabrous vines with panicles of pale yellow flowers, climbing over bushes, Santa Elena (11,104); La Libertad (11,306); Punta Centinela (11,389). Andersson (cf. Areschoug, p. 133) obtained it on Puná Island. The species was described originally from Vera Cruz, Mexico, and extends southward to the lowlands of Tumbez and Piura in Peru. This plant one would expect to find in the Galapagos Islands, but it is unknown there.

207. MENTZELIA ASPERA L. Sp. Pl. 516. 1753; Darlington, Ann. Missouri Bot. Gard. 21: 134. 1934. *Acrolasia squalida* Hook. f. Trans. Linn. Soc. 20: 222. 1847. *M. propinqua* Areschoug, Eugenies Resa Bot. 3: 133. 1910.

Rough sprawling annual with small orange-yellow flowers, on dry rocks or sometimes in underbrush. Punta Centinela (11,260); west base of Chanduy Hills (11,004); La Brea, Piura, Peru (H&S 11,599). The most widely-distributed member of the genus and extending from southern Arizona to northern Brazil and Bolivia. It was collected at Chanduy by Spruce (no. 6463), and also in the Guayaquil-Salinas area by Mille and by Hitchcock, but does not seem to be otherwise recorded from Ecuador. *M. propinqua* (from Puná Island) was said to differ from *M. aspera* in having acuminate petals much longer than the calyx, and the seeds fewer and tuberculate. Areschoug thought that Andersson's plant was especially close to *M. oligosperma* Nutt., from which it differed in its flattened, tuberculate seeds. Since Areschoug's comparisons were made from DeCandolle's Prodromus, it is easy to see where the difficulty lay, for DeCandolle described the petals as subrotund and obtuse and hardly longer than the calyx, and the seeds as 5 in a capsule and glochidiate only at the apex. In my Ecuadorean collections, the acuminate petals are 7 mm. long and 4 mm. wide, sepals 4–5 mm. long, and seeds 3 to 4 in a capsule. These specimens are identical with my collections (nos. 247 & 383) from the Galapagos Islands where the species is widely distributed on most of the islands, and with the collections cited by Darlington (l.c.) from the Ecuadorean mainland. In description of the species the petals are stated to be "4–8 mm. long, 2.5–4 mm. wide," and the calyx lobes "3–4 mm. long." What is meant by "outer stamens petaloid" (Darlington, p. 114) I have no idea. Flowers in the Ecuadorean collections closely resemble those illustrated in Addisonia 15: t. 504. 1930, from a Porto Rican plant.

208. M. FENDLERIANA Urban & Gilg, Mem. Torr. Bot. Club 3: 34. 1893; Darlington, Ann. Missouri Bot. Gard. 21: 117. 1934; Macbride, Fl. Peru, pt. 4: 145. 1941. An erect plant with orange petals 2

cm. long. Rocky slopes of Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,562). This is the locality near Talara where Haught made previous collections. He notes on his labels that this is one of the most beautiful plants of the region. It extends from Venezuela to Bolivia and is a plant characteristic of dry mountain areas rather than of the coast.

209. SCLEROTHRIX FASCICULATA Presl, Symb. Bot. 2, pt. 6: 4, t. 53. 1833; Macbride, Fl. Peru, pt. 4: 146. 1941. Annual with minute greenish flowers, on wet rocks, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,516). Mexico to Peru and Brazil; known from Albemarle, James, and Narborough Islands in the Galapagos.

CACTACEAE

210. CACTUS TOWNSENDII Britton & Rose, Cact. 3: 234. 1922. *Melocactus peruvianus* Vaupel in Engler, Bot. Jahrb. 50, Beibl. 111: 28. 1913; not *Cactus peruvianus* L., 1753. A melon-shaped cactus with small pink flowers, partially embedded in pure white wool interspersed with soft red spines. Rocky slopes of Cerro Prieto, Piura, Peru, 2000 ft. (H&S 11,383). The plant has previously been known only from Chosica, on the Lima and Oroya Railroad. It is the southernmost representative of the genus *Cactus*.

211. HYLOCEREUS aff. POLYRHIZUS (Weber) Britton & Rose, Cact. 2: 185. 1920. *Cereus polyrhizus* Weber in Schumann, Gesamtb. Kakteen 151. 1897. Climbing high over shrubs and trees, the branches trigonous to flattened. Fruit red, the exterior elongate-squamose, rotund-elliptic, about 12 cm. diam., dark purplish-red within, and of excellent flavor. Summit of Chanduy Hills, 1800 ft. (11,012). The shiny pear-shaped seeds are 2.5 mm. long. Ecuadorean collections made by Rose at the edge of the mangrove swamp near Guayaquil were tentatively assigned to this species by Britton & Rose. I saw no flowers, and there is no mention of the fruit in Ecuadorean collections.

212. LEMAIROCEREUS CARTWRIGHTIANUS Britton & Rose, Cact. 2: 100. 1920. Arborescent, 10 ft. high, the older growth with long shaggy spines, Puntilla, Salinas (11,199, in fruit) (Pl. 5, fig. 3). The trees are common in the dry bushy area west of Guayaquil, and on the Puntilla there are magnificent isolated specimens which have much the appearance of the arborescent *Cereus* of the Galapagos Islands (*Cereus Thouarsii*). The dull red edible fruit is 9–12 cm. long, nearly globular, and covered with a layer of weak spine-clusters (the individual spines about 3 cm. long). *Lemairocereus Cartwrightianus* was described as being 3–5 meters high and very common on the flats near Guayaquil; the tree, at least in the Salinas area, is so conspicuous that it could hardly have escaped the attention of such explorers as Tafalla (1800), Humboldt and Bonpland, or even earlier collectors who visited the area, then known as Peru. Collected in flower at 2000 ft. near summit of Cerro Prieto, Piura, Peru (H&S 11,341). The roseate bracted white flowers are 4–5 cm. long, with a short funnel-form tube, the lower part of which is thickly felted with lustrous brown pubescence.

213. OPUNTIA **melanosperma** sp. nov. Frutex semierectus 0.5 m. altus, ramis frequenter decumbentibus; articulis compressis, laete viridibus, obovatis (10–15 cm. longis, 7–9 cm. latis), areolis (2–5 mm. diam.) 2–4 cm. distantibus, cum pilis septatis opacis vel brunneo-translucentibus et glochidiis flavescentibus (2–5 mm. longis); spinis luteis rectis acicularibus (0.5–2 cm. longis), 1–3 in singulis areolis; floribus aureis deinde rubescentibus, ca. 6 cm. latis, petalis patentibus, obtusis (ca. 4 cm. longis); antheris flavis vel albidis (2 mm. longis), filamentis usque ad 2 cm. longis; ovario 3–4 cm. longo, non-spinoso, umbilicato; fructo 4 cm. longo, 2.5 cm. lato, rubescente, seminibus atris, curvulis (3 mm. longis, 2 mm. latis) minute tuberculatis cristatisque.

Shrub 2–4 ft. high, the flat joints with short yellow spines, flowers orange-yellow fading to dull pink. Beaches and sandy woods, Punta Ayangue (11,382, type in hb. Brooklyn Bot. Gard.) (Pl. 3, fig. 4). In all the species of *Opuntia* which I have examined the seeds are white with a practically smooth surface, and in Britton & Rose, The Cactaceae 1: 42. 1919, the genus is described as having seeds "covered by a hard bony aril, white, flattended." Though the vegetative growth is that of an *Opuntia* (the specimens were distributed as *Opuntia* aff. *elatior* Mill.) the seeds are much more like those encountered in the genus *Cereus*.

LYTHRACEAE

214. AMMANNIA AURICULATA Willd., Hort. Berol. 1: t. 7. 1806; Koehne, Pflanzenr. IV. 216: 45, fig. 5B. 1903. Annual with minute purple flowers, margins of pools east of Salinas (11,416, 11,161 in fruit); south of Engabao (11,000). The dichasia are only 3–5-flowered and the capsules only 2 mm. wide with rather short styles. The species is widely distributed from southern United States to Ecuador, and also in the Old World tropics.

215. CUPHEA CARTHAGENENSIS (Jacq.) Macbride, Field Mus. Bot. 8: 124. 1930. *Lythrum carthagenense* Jacq., Stirp. Am. 148. 1763. *C. balsamona* Cham. & Schlecht., Linnaea 2: 363. 1827; Koehne, Pflanzenr. IV. 216: 122. 1903.

Abundant in forest openings, Manglaralto (11,438). This is the small-leaved form (leaves 10–18 mm. long) identical with my collections from Indefatigable I., Galapagos. Schimpff's no. 97 from Indefatigable Island has much larger leaves (to 4.5 cm. long and 2.5 cm. wide) and the gland is less horizontal. Widespread in the tropics from Mexico southward.

216. ROTALA RAMOSIOR (L.) Koehne, in Mart. Fl. Bras. 13, pt. 2: 194. 1875. *Ammannia ramosior* L. Sp. Pl. 120. 1753. Annual with small magenta flowers and sessile capsules, pools east of Salinas (11,387). The immature capsules are somewhat

less rotund than is characteristic of var. *interior* Fernald & Griscom, Rhodora 37: 169. 1935, widespread in the United States. The collection is fairly close to *Stevens & Hess* 3894 from Guayama, Porto Rico. Koehne, Pflanzenr. IV. 216: 32. 1903, cites *R. ramosior* as having been found at Chanduy, Ecuador, quite evidently Spruce's collection. The plant is widespread from New York southward into the tropics, and is of scattered distribution in the Old World.

ONAGRACEAE

217. JUSSIAEA ERECTA L. Sp. Pl. 388. 1753; Munz, Darwiniana 4: 195, pl. 2, f. 1. 1942. Bushy plants 2–3 ft. high, with inconspicuous yellow flowers, margin of pool east of Salinas (11,419). The species is cited by Munz from the Guayaquil region, from Chatham Island, Galapagos Islands (*Schimpff* 138), and from Piura, Peru (*Haught* 171). It ranges from southern Florida to Bolivia and Paraguay, and is known from the Old World.

UMBELLIFERAE

218. EREMOCHARIS DISSECTA I. M. Johnston, Contrib. Gray Herb. 85: 174. 1929. A yellow-flowered plant growing in open rocky slopes, 1500 ft., Cerro Prieto, Amotape Hills, Peru (H&S 11,566). This is approximately the type locality; the source of Weberbauer's collection (7661) being Cerro Muerto, the next summit northward of the same ridge. It was also collected by *Haught* (no. 29), issued as *Eremocharis longiramea*.

219. ERYNGIUM FOETIDUM L. Sp. Pl. 232. 1753; Fawcett & Rendle, Fl. Jam. 5, pt. 3: 427, fig. 154. 1926. Abundant in openings of plantation, Manglaralto (11,483); a weedy species 1–2 ft. high, common in tropical America.

MYRSINACEAE

220. JACQUINIA PUBESCENS HBK., Nov. Gen. & Sp. 3: 251, t. 246. 1819. *J. mucronata* R&S. Syst. 4: 802. 1819. *J. Sprucei* Mez, Pflanzenr. IV. 236a: 43. 1903. A tree to 10 ft. high, with coriaceous dark-green leaves, and dull orange flowers, in fields northeast of La Libertad (11,195); ridge north of Santa Elena (11,373). The straight thick trunks of this tree and the nearly black evergreen foliage make the species outstanding in the landscape, where it often forms small groves on level land. Known locally as "barbasco" and the roots are dug for their insecticidal content. The poisonous fruit, as large as a small lemon, matures to a dull orange color (Pl. 8, fig. 6).

The description of *J. Sprucei* (based on *Spruce* 6478 from Chanduy, collections by *Andersson* and *Eggers* on Puná Island, and a *Ruiz & Pavon* specimen from "Peru: bei Cuenca") seems to have been quite unnecessary. When Mez treated *J. pubescens* in the Pflanzenreich (l.c.) he gave as locality: "Hylaea: am Ufer des Amazonenstroms ohne genaure Standortsangabe (Humboldt & Bonpland n. 3581)." The term "Hylaea" was used by Humboldt for the Amazon forest country in the sense of the word "selvas," but Mez included such widely-separated areas as Para, Santarem, and the Huallaga region of Peru. It is my experience that the best set of HBK specimens remained in Paris, and the Humboldt specimen cited by DeCandolle, Prod. 8: 150. 1844, "in Peruviae provincia Bracamorensi ad flumen Amazon, prope Cuenca ad littus Peruv." seems to give a more approximate statement of the locality. Humboldt and Bonpland, journeying southward from Loja, reached Ayavaca in northern Peru early in August, 1802, and proceeded to Huancabamba (also in the eastern part of Dept. Piura), and from there southeast to the Marañon, the great southern tributary of the Amazon. Here at Tomependa (junction of the Marañon and the Chinchipe) they repeated the experiments for determination of the length of a degree of latitude, which Condamine had made in 1737. From this point they went back across the mountains by way of Jaen and Cajamarca to Trujillo, and from there by land to Lima. After exploring the coast they left, in December, for Guayaquil en route to Acapulco, Mexico.

It will thus be seen that the type of *J. pubescens* probably came from eastern Piura; *J. mucronata* was apparently based on the same collection. Specimens from this region, which I have examined from the U. S. National Herbarium, appear to be identical with those which I collected on the coast of Ecuador. If descriptions of *J. Sprucei* and *J. pubescens* (as given by Mez) are compared, it will be seen that the differences are so slight as to be imaginary; the style in my Ecuadorean collections is 3 mm. long, though *J. pubescens* is supposed to be characterized by a "stylus subnullus." The distribution is not a strange one, since, except for littoral plants which extend along the coast to Lima, the chief mode of entry into the arid coastal area of Ecuador is through the dry mountain area extending back from Tumbez and Piura in Peru. It was of interest to see whether the Ecuadorean material was identical with any of the species from the north coast of Colombia, but all Colombian specimens appear quite different, being characterized especially by the fan-shaped striations of the calyx. Specimens from the Florida Keys (*J. keyensis*) and from Venezuela (*J. revoluta*) are very distinct from *J. pubescens* in the staminodia.

PLUMBAGINACEAE

221. PLUMBAGO SCANDENS L. Sp. Pl. ed. 2. 215. 1762; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 63. 1925. A vine-like shrub with white flowers, and calyx with long-stalked glands, summit of Chanduy Hills, 1800 ft. (11,046). The corolla tube is shorter (only 2 cm. long) than in most specimens examined, but otherwise the plant shows no difference from West Indian and Florida material. It is an abundant species of tropical America, and known from most islands of the Galapagos Archipelago, where it appears to be the same as the continental material.

APOCYNACEAE

222. THEVETIA PERUVIANA (Pers.) K. Sch. in Engler & Prantl, Nat. Pflanzenfam. IV. pt. 2: 159. 1895; Woodson, Bot. Maya Area [Carnegie Misc. Papers no. 15] 86. 1940. *Cerbera Thevetia* L. Sp. Pl. ed. 2. 304. 1762.

A tall shrub with large yellow flowers and brilliant red poisonous fruit, low ground between Ancon and Santa Elena (11,172). Widely planted as an ornamental in both hemispheres. It was grown in *Hortus Cliffortianus* under the supervision of Linnaeus, and was known at an early date from the West Indies, Mexico, and Peru.

223. VALLESIA GLABRA (Cav.) Link, Enum. Hort. Berol. 1: 207. 1821. *Rauwolfia glabra* Cav. Ic. 3: 50, t. 297. 1795. *V. cymbifolia* Ortega, Hort. Matr. Dec. 58. 1798. *V. dichotoma* R&P. Fl. Peruv. 2: 26, t. 151. 1799. *V. chiococcoides* HBK. Nov. Gen. & Sp. 3: 233, t. 241. 1819. *V. pubescens* Andersson, Eugenies Resa Bot. 195. 1855.

Vine-like bushes to 6 ft. high, abundant in the coastal region, with small glistening banana-shaped fruit. North slope of Puntilla, Salinas (11,109); Punta Ayangue (11,356). It was collected at La Libertad (as *V. dichotoma*) by Diels. (Fl. Ecuad., p. 125).

The minute greenish-white flowers appear very early in the season. On February 21, I collected specimens with mature fruit as well as flowers. My collections have the leaf-base rounded and therefore fit best into *V. dichotoma*, described from Ica on the Peruvian coast; *V. chiococcoides* came from Jaen de Bracamoros, Peru, therefore from the dry region of eastern Piura (cf. description of *Jacquinia pubescens*); *V. glabra* and *V. cymbifolia* are Mexican. From Lower California southward and from Ecuador to Argentina, the petioles are frequently hispid and the inflorescence more or less pubescent. An extreme is reached in the Galapagos Islands (*V. pubescens*) in which even the branches are short-pubescent, although perfectly glabrous plants and transitional forms are also found in these islands. Riley (Kew Bull. 1925: 224) considers *V. pubescens* as hardly more than a variety of *V. glabra*, and I agree with him. Material from the Florida Keys and the West Indies is always perfectly glabrous, with blunt leaves and longer carolla-lobes, and is a distinct species, *V. antillana* Woodson, Ann. Mo. Bot. Gard. 24: 13. 1937. The accompanying map (fig. 14) will show that *V. glabra* is characteristic of dry areas of the Pacific slope of Mexico and passes from Ecuador to the saline areas of western Argentina across the Peruvian and Bolivian Andes. The only collection that I have seen from Colombia is from "desert river bottom," Dagua Valley, *Killip & Hazen* 11,075. In this case it is evident that the Galapagos plant is to be associated with the western coast of America and not with the Bahamas (cf. *Euphorbia viminea*).

CONVOLVULACEAE

224. CALONYCTION PAVONII Hallier f. Bull. Herb. Boiss. 5: 1048. t. 17, f. 4–5; t. 18, f. 1. 1897. A white-flowered species climbing over bushes on sea-cliffs at the Puntilla, Salinas (11,216) (Pl. 17, fig. 3). Except for a collection (*March* 1133) from Jamaica which is cited by Hallier, it is restricted to the Guayaquil region (*Pavon;* Chanduy, *Spruce* 6948; between Guayaquil and Salinas, *Hitchcock* 20,037).

225. CRESSA TRUXILLENSIS HBK. Nov. Gen. & Sp. 3: 119. 1819; Kearney & Peebles, Fl. Plants Arizona, U.S. Dept. Agric. Misc. Publ. 423: 706. 1942. A white-leaved procumbent plant of saline places, collected from border of a pond at Salinas (11,277 & 11,160); cliffs at Punta Centinela (11,271); edge of salt marsh, Punta Ayangue (11,344). This species extends from southwestern United States to Peru. Strangely, it has not been reported from the Galapagos Islands.

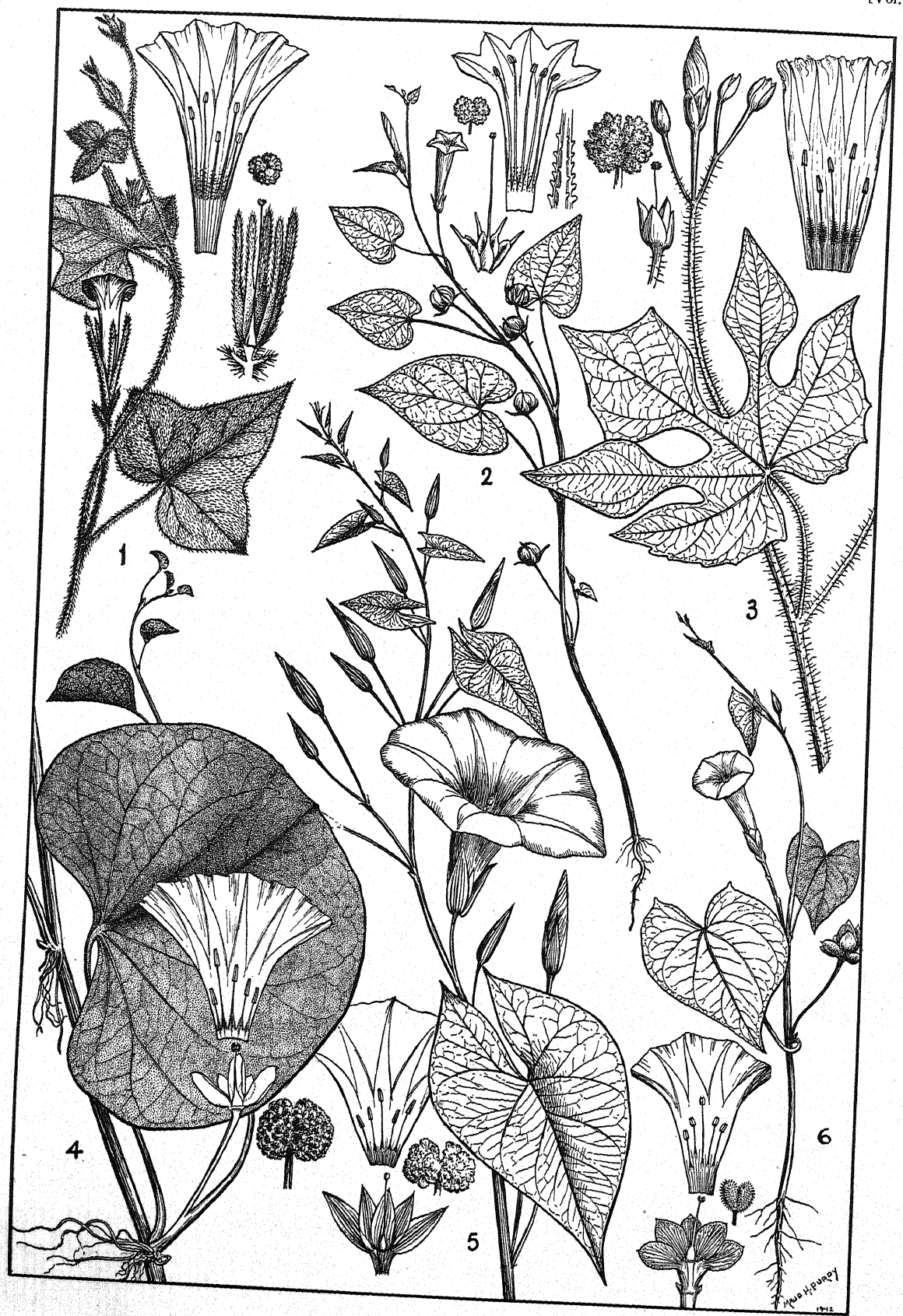
226. CUSCUTA HAUGHTII Yuncker, Mem. Torr. Bot. Club 18: 153. 1932. A parasite with reddish flowers common in sandy fields at Salinas (11,074, 11,281, 11,385). It grows on *Sida ciliaris, Aeschynomene pluriarticulata, Desmodium,* and grasses. The material is identical with that of *Haught* 154, which was obtained in Peru "6 miles east of Cape Pariñas," the only previously known collection.

227. EVOLVULUS GLABER Spreng. Syst. 1: 862. 1825; Van Ooststroom, Meded. Bot. Mus. Utrecht no. 14: 97. 1934. A sprawling blue-flowered herbaceous plant in dry ground at base of Chanduy Hills (11,021). A brownish-sericeous pubescence occurs sparsely on the stems and calyx of this widespread species, abundant in the Galapagos Islands, and extending from southern United States to Paraguay (fig. 14). It was collected on Puná Island by Andersson and by Sinclair.

228. E. PIURENSIS Van Ooststroom, Meded. Bot. Mus. Utrecht no. 14: 82. 1934. Rocky banks of Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,645). The entire plant is covered with sericeous pubescence and the flowers are much larger than in the preceding species. It has previously been known only from Haught's no. 28 and F 17 collected from the Pariñas Valley and Negritos, and from an old collection by Haenke without precise locality.

229. E. SIMPLEX Andersson, Eugenies Resa Bot. 211. 1855; Van Ooststroom, Meded. Bot. Mus. Utrecht no. 14: 122. 1934. An erect simple or branched plant with tiny white or bluish-tinged flowers in sterile ground on the Puntilla, Salinas (11,243 & 11,254); also on dry hills at the base of Cerro Prieto, Piura, Peru (H&S 11,552). It was originally described from the Galapagos Islands, but is known (cf. Van Ooststroom) from several collections in Peru: Dept. Tumbez, Zorritos, *Weberbauer* 7742; Dept. Piura, Toblazo, *Haught* F 144 and 218; Prov. Piura, Serran, *Weberbauer* 5980; Dept. Cajamarca, Cascas, *Raimondi* 7577.

230. IPOMOEA AEGYPTIA L. Sp. Pl. 162. 1753; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 111. 1925. *Convolvulus pentaphyllus* L. Sp.


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Pl. ed. 2, 233. 1762 (Pl. 3, fig. 3). Twining white-flowered annual with 5-foliate leaves, widely distributed in American and Old World tropics; among rocks on Puntilla, Salinas (11,220) and on river gravels, near La Brea, Piura, Peru (H&S 11,613). Abundant in the Galapagos Islands.

231. I. CARNEA Jacq., Enum. Pl. Carib. 13. 1760; Diels, Fl. Ecuad. 127. 1937. A vine-like shrub with pink flowers and soft cordate leaves common in the scrub growth, Ancon (11,174); Punta Ayangue (11,352). It was not in flower January-April, but the long-haired seeds were conspicuous at this time. Dry regions of West Indies, Central America, the coast of Venezuela and Colombia, and northwestern Peru.

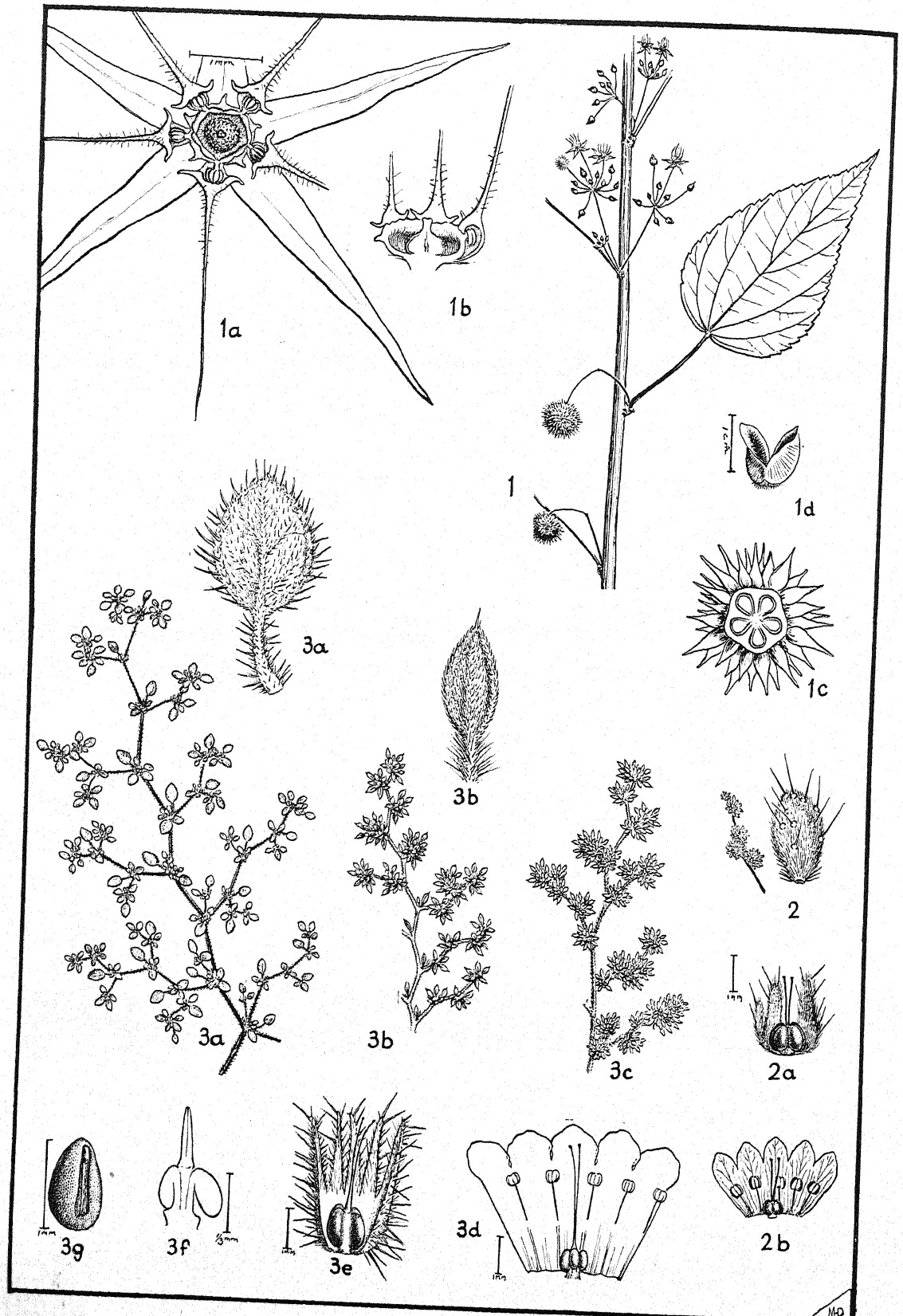
232. I. CHILENSIS A. Br. & Bouché, Ind. Sem. Hort. Berol. Append. 1. 1857; I. M. Johnston, Contrib. Gray Herb. no. 85: 95. 1929. *I. paposana* Philippi, Flor. Atacam. 36. 1860. Upright or trailing plants on rocky slopes of Cerro Prieto, 1500 ft., Piura, Peru (H&S 11,510) (Pl. 17, fig. 2). The flowers are small (2 cm. long), dark red, often on plants only 2 dm. high. These plants are similar to *Johnston* 5311 from Dept. Taltal in northern Chile and to the Peruvian collections represented by *Rose* 18,621 from Santa Clara and *Weberbauer* 5231 from Hills of Mongomarca near Lima. The stamens are scarcely exserted and the flowers seem to be a little smaller and with less-spreading corolla-limb than in *Ipomoea coccinea*. However, the seeds are identical with those of *I. coccinea* in being partially covered with an appressed sericeous-brown pubescence. If *I. chilensis* is to be maintained as a species distinct from *I. coccinea* L., the name should probably give way to *I. angulata* Ortega Dec. 7: 8. 1798 (?); Ruiz & Pavon, Fl. Peruv. 2: 11, t. 120, f. 6. 1799, which was described from rocky elevations near Lima. This plant was renamed *Quamoclit Ruiziana* Don. Gen. & Syst. Pl. 4: 258. 1838. The descriptive term "angulatus" applied to the stem, and the remainder of the description, except the dubious term "subtuberculatae" referring to the calyx-lobes, precisely fit *Ipomoea chilensis*.

233. I. CRASSIFOLIA Cav. Desc. 100. 1802. Without question the white-flowered plant with erect stems and thick reniform leaves which Cavanilles grew from seeds obtained from Guayaquil. It forms large patches, with branches sometimes trailing, from La Libertad to the eastern edge of Salinas, and is especially abundant in the village of Moya, east of Salinas (11,413). It is also known from the vicinity of Talara, Peru (*Haught* 210, in which he notes that it is "a trailer in sandy soil back of the beach, and also in water-courses in the Pariñas Valley at least thirty miles inland"). Specimens from the latter location root at the nodes (H&S 11,513, as illustrated in Pl. 17, fig. 4), but such a condition is perhaps due to previous inundation. In the Gray Herbarium is an old specimen (Cuming's Herb. 975) from "Lima or the north of Peru." *I. crassifolia* is probably "I. urbica" cited in Bentham, Bot. Sulphur (p. 135) from Guayaquil. There is much similarity between this plant and specimens from the orient such as *C. O. Levine* 1208 (B) from Honan Island near Canton, China (issued as *I. reptans* Poir.).

234. I. aff. DUMETORUM (HBK.) Willd. ex R&S. Syst. 4: 789. 1819. *Convolvulus dumetorum* HBK. Nov. Gen. & Sp. 3: 101. 1819. A glabrous annual with small flowers only 2 cm. long. These are white, usually with a purplish center and corolla stripe, but sometimes the entire corolla is faintly tinged with blue. It grows in dry rocky or gravelly soil, and the inflorescence is most frequently 1-flowered (as in the illustration, Pl. 17, fig. 6) but not infrequently 2- or 3-flowered as in *Haught* 55a. Collections were made on the Puntilla, Salinas (11,225) and in river gravels, Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,614). The illustration shows a small plant in which the angles of the leaves are only slightly developed. The plants may be either erect or long-twining. The large seeds (7 × 4 mm.) are covered with long gray tomentum, and the septum of the capsule is translucent in the manner of *Lunaria annua*. This plant was also collected by Haught at Talara, nos. 55a and 142; it probably represents a distinct species, characteristic of this desert coast, but with so many of the old collections at present unidentified, I hesitate to introduce a new name. The specimens agree fairly well with photographs of *I. dumetorum*, but are probably nearer to var. *glaucescens* (HBK.) Choisy is DC. Prod. 9: 378. 1845, which is described as having shorter 1–3-flowered peduncles. Both of these species came from the mountains: *I. dumetorum* from Quindiu, Colombia, at 4200 ft.; and *I. glaucescens* from Chilo, near Quito, at 8000 ft. Neither of these localities ordinarily have elements of the coastal flora.

235. I. INCARNATA (Vahl) Choisy in DC. Prod. 9: 361. 1845; Boldingh, Fl. Nederl. W. Ind. 331. 1913. *Convolvulus incarnatus* Vahl Ecl. 2: 12. 1798. *I. linearifolia* Hook. f. Trans. Linn. Soc. 20: 204. 1847. *I. Kinbergi* Anderss., Eugenies Resa Bot. 212. 1854. This species, with large white flowers having a purple center and broad purple line in corolla-lobes, is an abundant trailing plant on the Puntilla at Salinas (11,217) where it grows with *Portulaca Haughtii* within the range of salt spray (Pl. 17, fig. 5). I also collected it on sandy roadsides at Salinas (11,288), on sea-cliffs at Punta Centinela (11,403), and on river gravels near La Brea, Peru (H&S 11,624). It has been known previously from Piura (Talara, *Haught* 221, and

Plate 17. Corolla × ½; stigmas × 5, except *I. Nil* × 7.5.—Fig. 1. *Ipomoea Nil* (11,219, Ecuador).—Fig. 2. *Ipomoea chilensis* (*Haught & Svenson* 11,510, Peru).—Fig. 3. *Calonyction Pavoni* (11,216, Ecuador).—Fig. 4. *Ipomoea crassifolia* (11,513, Ecuador).—Fig. 5. *Ipomoea incarnata* (11,217, Ecuador).—Fig. 6. *Ipomoea* aff. *dumetorum* (*Haught & Svenson* 11,614, Peru).


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1b
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1d
1c
3a
3a
3b
3b
3c
2
2a
3g
3f
3e
3d
2b

Nomala, *Weberbauer* 5927), and from Cancas, Tumbez, *Weberbauer* 7755 (US).

I. incarnata came originally from Curaçao and it is known also from Aruba, Bonaire and the adjacent coast of Venezuela. In the Galapagos Islands the species is very abundant, occurring usually with much reduced leaves (cf. Amer. Jour. Bot. 22: 249. 1935) as *I. linearifolia*. Riley (Kew Bull. 1925, p. 226) notes the collection of *I. linearifolia* at the base of a crater two miles inland on Indefatigable Island, a locality which cannot be far from the original station for *I. Kinbergi*. By Hallier, in Engl. Bot. Jahrb. 16: 555. 1893, *I. Kinbergi* was reduced to synonymy under *I. incarnata*, and *I. galapagensis* under *I. triloba* (op. cit. 18: 138. 1894). *Ipomoea incarnata* thus represents a species of the arid north shore of South America, the Galapagos Islands, and the arid regions at the entrance of the Gulf of Guayaquil (fig. 15). Like many other species it shows marked reduction of leaf-surface in the Galapagos Islands, where it survives in the driest of habitats.

236. I. Nil (L.) Roth, Cat. 1: 36. 1797; Hallier f. in Engler Bot. Jahrb. 18: 136. 1894; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 112. 1925. Among rocks, north slope of Puntilla, Salinas (11,219); river gravels, near La Brea, Piura, Peru (H&S 11,219) (Pl. 17, fig. 1). The appressed pubescence on the upper leaf-surface of no. 11,219 is so thick as to be almost velvety, and the tips of the calyx lobes are a little wider than usual in this species. It is exactly the plant represented by *Snodgrass & Heller* 418 & 511 from Charles Island, and by *A. H. Hitchcock* 20,033, from "rolling scrubland" between Guayaquil and Salinas. *Baur* 200 from Chatham Island is even more hairy. This blue-flowered species is of scattered distribution in the tropics of both hemispheres, and varies considerably in the amount of pubescence, especially of the calyx lobes. The pubescence is consistently composed of firm strigose yellow hairs set at right angles to the calyx-tube. This form with hairy calyx projections differs from many of the old illustrations of *I. Nil*.

237. I. Pes-caprae (L.) Sweet, Hort. Suburb. Lond. 35. 1818. *Convolvulus Pes-caprae* L. Sp. Pl. 159. 1753. The flowers of this widespread strand plant are here (Salinas, 11,198) unusually small, only 2.5 cm. wide (Pl. 3, fig. 2); in this respect they are like collections from the Galapagos Islands (*Stewart* 3127 & 3126; *Snodgrass & Heller* 125) in the Gray Herbarium. It probably represents a dwarfed variation characteristic of the Galapagos Islands and the adjacent coast. Material examined from Florida and the West Indies has flowers 4–5 cm. wide, and Hallier in his detailed description (Bull. Herb. Boiss. 5: 376. 1897) states that the corolla is 4 cm. long and 6.5 cm. wide.

238. I. aff. tiliacea (Willd.) Choisy in DC. Prod. 9: 375. 1845; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 116. 1925. *Convolvulus tiliaceus* Willd. Enum. 1: 203. 1809. This plant is represented in various forms in the vicinity of Guayaquil and in northern Peru. For the most part the leaves are thin, strongly cordate, and acuminate (as in *Weberbauer* 6434 (US) from above Chulucanas, Piura, Peru, a collection with rather elongate calyx-lobes, and my collection from damp roadsides at Manglaralto (11,454)). This phase passes to pubescent plants in which the hispid ciliation covers much of the surface of the outer calyx bracts, and in which the leaves are incipiently trilobed, as in *Hitchcock* 20,068 (US), from Oil Camp between Guayaquil and Salinas. Further toward the coast, as at Baños San Vicente (11,499), the leaves are deeply three-parted, and the pubescence just as in Hitchcock's collection. A collection of the same plant, *Stork, Eyerdam, & Beetle* 8965 (US) from 8 km. north of Guayaquil, shows somewhat thicker leaves, similar coriaceous sepals varying from pubescent to glabrous (the innermost longer and obovate), a pubescent capsule, and glabrous seeds. A similar plant with larger leaves was collected by Eggers (14,883) from Balao. In this last-mentioned collection the entire leaves are as much as 9 cm. across, and it seems identical with material from northwest of Bogota, Colombia, *A. E. Lawrence* 59 (US) which has been labeled as *I. denticulata* (Desr.) Choisy. The color of the corolla varies from purplish or pink to white (with a red or purple throat), as mentioned by Britton & Wilson for *I. tiliacea*.

239. Jacquemontia corymbulosa Benth., Bot. Sulphur 137. 1845. An abundant vine with deep blue flowers climbing over bushes, Manglaralto (11,442). This is the same as *Jameson* 596 (US) "From the cerro of Santana, Guayaquil—Flowers sky-blue, May 1846"; which in all characters fits Bentham's description, the plant coming from Guayaquil. It is a rather slender species, with tawny pubescence prominent on the unfolding leaves and about the calyx, but with glabrous mature leaves; and apparently confined to western Ecuador, being similar to *J. pentantha* (Jacq.) G. Don, but with the corolla much shorter. The seeds are prominently roughened.

240. J. nodiflora (Desr.) G. Don, Gen. Syst. 4: 283. 1838. *Convolvulus nodiflorus* Desrouss. in Lam. Encyc. 3: 557. 1791. A tomentose vine with small white flowers, described from Santo Domingo

Plate 18.—Fig. 1. *Buettneria glabrescens* (11,126, Ecuador); branch with flowers and fruit, and enlarged flower (1a); side view of flower (1b) showing filiform petals with expanded hooded bases which protect stamens (attached to crateriform staminal tube); enlarged cross-section of immature fruit (1c), and a mature carpel (1d).—Fig. 2. *Coldenia Darwini* (*Howell* 10,060, Galapagos I.); fragment of branch and a single leaf × 4; group of 4 nutlets within calyx (2a), and a corolla (2b).—Fig. 3. *Coldenia paronychioides*, from Ecuador, showing natural variation; a branch (3a) of 11,210, the abundant form, with enlarged leaf × 4; (3b) a narrow-leaved form (11,214); (3c) a form with congested leaves (11,606). Corolla (3d), nutlets (3e), ovules (3f), and seed (3g) (all from 11,428) are shown in detail.

(Haiti) and extending southward in the tropics; Chanduy Hills, 1800 ft. (11,060). This material differs greatly from that in the West Indies, as represented by *Leonard* 11,082 and similar collections. No. 11,060 has larger acuminate leaves (to 8 cm. long), petioles frequently 2.5 cm. long, as long as the peduncles of the 10–50-flowered cymes. The corollas, on the other hand, are small, only 1.0 cm. long as compared with 1.5 cm. in West Indian plants, but the details of structure are the same. It is the same as *Hitchcock* 17,992 (US) from Guayaquil, and the species was reported from Guayaquil by Bentham, Voy. Sulphur, p. 138.

241. J. SECUNDA (Ruiz & Pavon) Choisy, Mém. Soc. Phys. Genève 8, pt. 1: 62. 1838. *Convolvulus secundus* Ruiz & Pavon, Fl. Peruv. 2: 10. 1799. A low arching, vinelike shrub with silvery leaves and bright blue flowers. It is very abundant on the sea-cliffs at Ancon and also on the Puntilla at Salinas. Collections were also made on the hills north of Punta Carnera (11,292) and at Quebrada Ancha, near La Brea, Peru (H&S 11,570). Haught collected this plant from La Brea on March 31, 1921, and from Talara (no. 37) in 1925. It blooms late in the rainy season and is one of the few wild plants colorful enough to make any impression on the local inhabitants. I have seen it used as a vine on houses at Salinas. Haught (in a note accompanying no. 37) remarks that it is "one of the finest of the native wild flowers here." This species is apparently confined to the dry coast of northwestern Peru and western Ecuador.

HYDROPHYLLACEAE

242. NAMA DICHOTOMUM (Ruiz & Pavon) Choisy, Mém. Soc. Phys. Genève 6: 113. 1833; Hitchcock in Amer. Jour. Bot. 20: 528. pl. 27, fig. 1, 29. 1923. Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,550). Known also from the Galapagos Islands: Albemarle I., Tagus Cove (*Stewart* 3144—cited by Hitchcock). A widespread annual from Colorado and New Mexico southward to Peru and Chile.

BORAGINACEAE

243. COLDENIA PARONYCHIOIDES Philippi, Cat. Itin. Tarapaca 55. 1891; I.M. Johnston, Contrib. Gray Herb. no. 70: 60. 1924. Prostrate tomentose plants with minute pale blue to white flowers, abundant on sandy flats and dunes, north and south of Salinas (11,162, 11,163, 11,210–11,215, 11,428); exposed sea cliffs, Puntilla, Salinas (11,080); dunes, Punta Centinela (11,268); dunes, Negritos, Piura, Peru (H&S 11,606). *Coldenia* is one of the most interesting genera on the Galapagos Islands, and since the plants at Salinas showed a diversity in compactness, width of leaves, color of flowers, and obtuseness of corolla lobes, I made many critical collections to correlate variations, if possible, with species of the Galapagos Islands. All of these plants turned out to be *C. paronychioides,* known previously from northern Peru to Chile.

The seedlings (cf. 11,162, 11,211) form regular geometric patterns, but older plants assume various degrees of compactness as the branches sprawl out from a perennial rootstock. Individual plants may become more than two feet in diameter. These variations are shown by Miss Purdy in Pl. 18, fig. 3. They are quite evidently of an individual nature, and of no significance in the species problem. *C. paronychioides* is closely allied to the Galapagos species and the North American *C. hispidissima, C. tomentosa, C. mexicana, C. Purpusii,* and *C. cuspidata*—these form the section *Eddya,* characterized by rather small nutlets, not closely crowded, and with a ventral groove (cf. Johnston, p. 56 and 57). Howell more recently (Proc. Calif. Acad. Sci. ser. 4, 22: 99–110. 1937) treats the Galapagos material as representing four species. *C. paronychioides* is closest to *C. Darwinii* of the Galapagos, but, as may be seen from the illustration of that species (Pl. 18, fig. 2), the relationship is not very close, for *C. Darwinii* has much smaller fruit, styles parted to the base, and anthers attached at the base of the perianth tube. Both *C. Darwinii* and *C. paronychioides* have smooth shiny black seeds, with very minute reticulation; in both species hairs with bulbous bases occur on the leaves.

244. CORDIA BIFURCATA R&S. Syst. 4: 466. 1819. Shrub with minute orange flowers, near sea level, Manglaralto (11,477, det. Johnston). Type locality: Chacahuassi, Peru.

245. C. LUTEA Lam. Ill. 1: 421. 1791; *C. rotundifolia* R&P. Fl. Peruv. 2: 24, t. 148a. 1799. Dwarf shrubs with large yellow flowers, in the windswept part of the Puntilla often flowering when less than a foot in height (11,155). River gravels near La Brea, Piura, Peru (H&S 11,596). This bush or small tree has the most conspicuous flowers to be seen in the Galapagos Islands, where it is a common species. Likewise on the coast of Ecuador and Peru it is a prominent feature of the landscape. It was originally collected in Peru by Dombey at "Huanxa & aux environs de Lima," and is confined to the drier parts of Ecuador and Peru (cf. Johnston, Jour. Arn. Arb. 26: 44. 1935). Diels (Fl. Ecuad., p. 127) cites it from Chanduy (*Spruce*), Guayaquil (*Hartweg, Diels*), El Recreo (*Eggers*), and from Tumbez, Peru.

246. C. POLYANTHA Benth., Pl. Hartweg. 121. 1843. *C. guayaquilensis* DC. Prod. 9: 496. 1845. A pubescent shrub with white flowers in prominent rounded heads, summit of Chanduy Hills, 1800 ft. (11,011); ridge north of Santa Elena (11,142, 11,381); Punta Ayangue (11,350); on disintegrated granite, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,523). This species (both names based on the *Hartweg* collection from Guayaquil), was also seen in savannas east of Guayaquil by Diels (Fl. Ecuad., p. 128). He states that its distribution is similar to that of *C. lutea,* but extending farther to both north and south, and cites material from Boca Grande, southwest Colombia, from Jipijapi (*Warscewicz*) in Manabi Province, from Guayaquil and Puná Island, and from Piura, Lambayaque, and

Lima, Peru. It is not known from the Galapagos Islands, though the resemblance to narrow-leaved specimens of the smaller-flowered *C. Scouleri-C. leucophlyctis* group is exceedingly close. There is great variation in types of pubescence on the leaves and calyx of *C. polyantha*—just as in the Galapagos group just mentioned. In material collected at Guayaquil by Schimpff during the dry month of December the pubescence of the unfolding leaves consists of closely packed stellate scales. In the material which I collected at Santa Elena during a wet period (March, 1941) the stellate and single hairs are greatly elongate, and forked hairs occur on some of the specimens.

247. HELIOTROPIUM ANGIOSPERMUM Murray, Prodr. Stirp. 217. 1770. *H. parviflorum* L. Mant. 2: 201. 1771. A weedy perennial (sometimes annual) with small white flowers, the spikes usually 2 (sometimes 3) together, in thickets of *Cordia lutea,* Salinas (11,153); rocky slopes of Cerro Prieto, Piura, Peru (H&S 11,602). This is a widely distributed plant from Florida and the West Indies to South America, and is found on most of the Galapagos Islands.

248. H. CURASSAVICUM L. Sp. Pl. 130. 1870; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 135. 1925. Annual with fleshy leaves and small white flowers abundant on salt flats, Salinas (11,197). One of the commonest plants on tropical coasts of both hemispheres. This collection does not show the brilliant violet coloration of the leaves so frequently seen after drying.

249. HELIOTROPIUM **piurense** Johnston, sp. nov. Herba gracilis erecta 1–2 dm. alta e radice annua palari ad 1.5 mm. crassa oriens; caule basi simplici 1–1.5 mm. crasso, supra ascendenter dichotomoramosissimo, pilis pallidis rigidulis gracilibus falcatocurvatis antrorse adpressis 0.3–0.4 mm. longis sparse vestito; internodiis 0.5–3 cm. longis; foliis 1–1.5 cm. longis numerosis caulinis (inferioribus sub anthesi deciduis) oblanceolatis sparse strigosis (pilis rigidulis minutis non rariter e pustulis minutis orientibus) supra medium 2–3 mm. latis deinde basem versus in petiolum gracilem 1–2 mm. longum gradatim contractis, apice acutis, margine laxe subrevolutis, subtus pallidioribus costatis sed enervatis; spicis terminalibus gracilibus 5–10 (rariter ad 15) cm. longis foliosis maturitate laxifloribus; calyce sparse strigoso; lobis lanceolatis vel late lanceolatis acutis inaequalibus, juventate 2–2.5 mm. longis erectis, fructiferis 3–3.5 mm. longis ascendentibus; pedicellis brevibus, sub anthesi ad 0.3 mm. longis, fructiferis ad 0.5 (vel rariter ad 1) mm. longis; corolla alba inconspicua cylindrica 2.4 mm. longa elongata extus sparse strigulosis intus glabra, lobis oblongis erectis vel stricte ascendentibus ca. 0.8 mm. longis et 0.3 mm. latis lobos calycis paullo superantibus; antheris ca. 0.7 mm. supra basem tubi corollae affixis ca. 0.5 mm. longis basi rotundis apice in appendiculatum brevem contractis coherentibus; filamentis gracilibus ad 0.1 mm. longis; ovario glabro; stigmate late conico ad 3 mm. diametro et alto subsessili; nuculis 4 ca. 1 mm. alto, ventre angulatis perforatis, dorse alte convexis pilis gracilibus erectis vel ascendentibus uncinatis gerentibus.

Peru, Dept. Piura: Cabo Blanco, April 14, 1929, *Haught* 256 (G); base of Cerro Prieto, dry hills, 400 ft. alt., March 28–30, 1941, *Haught & Svenson* 11,553 (Type, Gray Herb.).

A very distinct and well marked species which keys out to *H. ocellatum* Cham. in the monograph of the South American species of Heliotropium, Johnston, Contr. Gray Herb. 81: 49 (1928), and probably has its closest relative in that species of Uruguay. It differs from that species, however, in its much more slender and erect habit, smaller flowers, and uncinate hairs on the smaller fruit. From the weedy *H. fruticosum* L., of northern South America and the West Indies, which also has uncinate hairs on its fruit, *H. piurense* is quickly distinguished by its subsessile stigma and very much more slender habit. It grows inconspicuously among sparse grasses on dry soil. (Locality in foreground of Pl. 5, fig. 6.)

250. H. RUFIPILUM (Benth.) I.M. Johnston, Contrib. Gray Herb. no. 81: 44. 1928. *Tournefortia rufipila* Benth. Bot. Sulphur 140. 1844. A shrub with soft lax acuminate leaves (to 15 × 5 cm.) with terminal inflorescences of minute white flowers and small dry fruit, along river bank, Manglaralto (11,469). This is the typical form of the species, which was described from neighboring Salango. Between this material and the single known collection from the Galapagos Islands—from the dry northwest coastal region of Charles Island (*Schimpff* 200, hb. Brooklyn)—there is the great variability associated with *Lantana, Waltheria,* and many other genera in the Galapagos Islands. Both of these collections have been determined by Johnston as var. *genuina,* in which the nutlets have long-stiped glands. Except for a collection by Spruce at Chanduy (no. 5983), *H. rufipilum* does not seem to occur in the dry outer zone of the Ecuadorean coast. It extends to Piura, Peru, and to an outlying station at Yungas, near La Paz, Bolivia (*Rusby* 1437). To quote from Johnston, "The typical form of the species is known from San Salvador, Guatemala, and southern Mexico, where it is confined to the Pacific slope. It reappears in Ecuador, but seems to be much less common there than the endemic South American variety, *anadenum.*" According to Johnston, *H. rufipilum* is very close to the Andean *H. Urbanianum.*

251. TOURNEFORTIA BICOLOR Sw. Prodr. 40. 1788; Britton & Wilson, Sci. Surv. Porto Rico and Virgin Isls. 6: 132. 1925. A shrub 10 ft. high, with small white flowers and white pulpy fruit. The leaves are glabrous throughout; river bank, Manglaralto (11,459). A widely distributed shrub or stout vine from the West Indies into tropical America. This is the typical form of the species in which the calyx lobes do not exceed 2 mm. in length. Var. *calycosa* is reported by Johnston, Jour. Arn. Arb. 16: 52. 1935, as occurring in Ecuador.

252. T. PSILOSTACHYA HBK. Nov. Gen. & Sp. 3: 78. 1818. *T. strigosa* Andersson, Eugenies Resa Bot. 207. 1855. A straggling shrub (usually about 5 ft. high) with minute orange corollas, followed by slightly pulpy fruit, ridge north of Santa Elena (11,141); Punta Ayangue (11,339). These specimens are identical with collections of *T. strigosa* which I made in the Galapagos Islands; Killip also considers the two species as the same. *T. psilostachya* was described from "littore oceani Pacifici prope Guayaquil," a locality perhaps in the vicinity of Santa Elena. It seems to be confined to the Galapagos Islands and the adjacent coast of Ecuador.

VERBENACEAE

253. AEGIPHILA SCHIMPFII Moldenke, Rep. Spec. Nov. 49: 117. 1940. Shrubs 6–9 ft. high; flowers yellow with exserted stamens. Along stream, Manglaralto (11,448) (det. Moldenke).

254. BOUCHEA FLUMINENSIS (Vell.) Moldenke, Phytologia 1: 265. 1937. *Verbena fluminensis* Velloso, Fl. Flum. 1: t. 38. 1827. Herbaceous plant 2–3 ft. high, with purplish-pink flowers, common in open places, Manglaralto (11,498) (det. Moldenke). The species was described from Brazil.

255. CITHAREXYLUM sp. (det. Moldenke). Shrub 6–8 ft. high, on river banks, Manglaralto (11,457), with very large leaves up to 15 × 8 cm.

256. CLERODENDRON MOLLE HBK. Nov. Gen. & Sp. 2: 244. 1818. Shrubs 5–10 ft. high, forming thickets in moist low ground between Ancon and Santa Elena (11,173). The pinkish flowers in terminal cymes are followed by corky capsules which are well adapted for floating. The material agrees with the original description of specimens coming from Guayaquil except that the upper leaf surface is a little pubescent. It has a densely pubescent under-surface of the leaf and is the same as plants which I collected on Charles Island in the Galapagos (no. 156). Specimens identical with var. *glabrescens* Svenson, Amer. Jour. Bot. 22: 251. 1935, were collected on Indefatigable Island by Schimpff (no. 70) and named *C. ternifolium* HBK at Berlin. These glabrous and pubescent plants are evidently trivial forms of the same species. They have aculeate petiole bases and are not far removed from the West Indian *C. aculeata.*

257. CORNUTIA ODORATA (Poepp. & Endl.) Poepp. ex Schauer in DC. Prod. 11: 681. 1847; Moldenke, Rep. Spec. Nov. 40: 176. 1936. *Hosta odorata* Poepp. & Endl. Nov. Gen. & Sp. 3: 63, t. 269. 1845. Bushes to 7 ft. high, the large panicles of pale violet flowers with a strong lavender smell; banks of stream, Manglaralto (11,455) (det. Moldenke). The species was described from "prov. Maynas ad Yurimaguas," Peru.

258. LANTANA SPRUCEI Hayek, in Fedde, Rep. Spec. Nov. 2: 162. 1906. Suberect or prostrate (rarely erect) shrubs with white to pale purplish flowers with yellow center, abundant on rocky slopes, dunes and sea cliffs, Puntilla, Salinas (11,127); ridge north of Santa Elena (11,147, 11,371, 11,376, 11,650); Punta Centinela (11,406, 11,259); Manglaralto (11,490); base and slopes of Cerro Prieto, Piura, Peru (H&S 11,619, H&S 11,575); Quebrada Mogollon, Amotape Hills, Peru (H&S 11,536). These specimens have obtuse to acute leaves and appressed to spreading pubescence, but all appear to represent the same species. Hayek, describing the pubescence of *L. Sprucei,* based on *Spruce* 6517 from Chanduy, says "folia . . . . in pagina superiore setulis minimis scabrida, in pagina inferiore setulis minimis scabrida et pube brevi hirtella," which in my estimation well describes the specimens of my collection. This is a very abundant plant along the dry coast.

L. pedunculata of the Galapagos Islands is a close relative of *L. Sprucei,* but appears to be quite different in its strongly hispid pubescence, especially of the corolla tube, its short corollas (8–9 mm. long as compared with 11–14 mm. in *L. Sprucei*), the somewhat longer style (2.2 mm. as compared with 2 mm. in *L. Sprucei*), and its more deeply channeled fruitstones. Hayek compared *L. Sprucei* with the Mexican *L. hispida* HBK., which appears to be distinct in its short peduncles, smaller flowers, and pilose bracts.

259. LIPPIA CANESCENS Kunth, Nov. Gen. & Sp. 2: 263. 1818. *Phyla canescens* Greene, Pittonia 4: 48. 1899. Forming mats on cliffs in salt spray, Puntilla, Salinas (11,081); on dunes, Salinas (11,164). Only rarely do the leaves reach 1 cm. in length. The flowering heads are only 3–4 mm. in diameter, and the corollas are also very small (1–1.5 mm. long) and the mature fruit is black-punctulate. The prostrate woody stems form an intricately-branched mat a foot or two in diameter. These plants have essentially the same flower and fructification as *Lippia asperifolia,* and the presence of Malpighiaceous (2-armed) hairs on the leaves and stem would seem to be the only distinction for the separation of the genus *Phyla* from Lippia. Such hairs are found in a very large number of families (cf. Solerader, Systematic Anatomy of Dicotyledons (ed. Scott) 2: 1117. 1908), and in themselves should offer no basis for generic distinction.

260. L. LANCEOLATA Michx. Fl. Bor.-Am. 2: 15. 1803. Robinson (Contrib. Gray Herb. no. 45: 532. 1916) has referred all the Galapagos material to *L. reptans* HBK. with the notation that it is "a species fairly well marked as to its firmer veins and more salient teeth of the leaves." Whatever may be the status of other collections from these islands, my no. 255 from Indefatigable Island is in no way separable from collections of *L. lanceolata* from the United States, such as *Deam* 5290 from Indiana or *Emig* 124 from Missouri. These all represent the broad-leaved phase of the species (var. *recognita* Fernald & Griscom, Rhodora 37: 178. 1935).

261. STACHYTARPHETA sp. (det. Moldenke). Herbaceous plant with large reddish-purple flowers 1–2 cm. long in narrow spikes, fields at west base of Chanduy Hills (11,003).

LABIATAE

262. HYPTIS MUTABILIS (Rich.) Briquet in Bull. Herb. Boiss. 4: 788. 1896. *Nepeta mutabilis* Rich. Actes Soc. Hist. Nat. Paris 110. 1792. *Hyptis spicata* Poit. Ann. Mus. Paris 7: 474. 1806. Moist forest bordering plantations, flowers pale lavender, Manglaralto (11,446). Widespread in the American tropics.

263. H. PECTINATA (L.) Poit. Ann. Mus. Paris 7: 474. 1806; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 159. 1925. *Nepeta pectinata* L. Syst. ed. 10, 1097. 1759. Sandy beach, Manglaralto (11,465 & 11,493). Abundant in tropics of both hemispheres.

264. MARSYPIANTHES CHAMAEDRYS (Vahl) Kuntze, Rev. Gen. 524. 1891; Britton & Wilson, op. cit., p. 160. *Clinopodium Chamaedrys* Vahl, Symb. 3: 77. 1794. *Marsypianthes hyptoides* Mart. ex Benth. Lab. 64. 1832; Hook. Ic. t. 457. 1842. An annual, or infrequently perennial (cf. 11,154) with insignificant blue flowers in pedunculate heads, and seeds closely resembling those of *Allionia incarnata;* sandy flats, headlands, and rocky slopes, Salinas (11,130, 11,245, 11,154); Punta Centinela (11, 396); Quebrada Mogollon, Amotape Hills, Peru (H&S 11,533). Areschoug (p. 128) notes that the specimens collected by Andersson on Puná Island differ from the Hartweg collection in having shorter and less erect stems, with longer oblong-ovate leaves. Hooker (l.c.) notes the plant as "A very common weed, especially near the sea, in the greater part of tropical America, from Mexico to Guayaquil on one coast, and to South Brazil on the other." It is not recorded from the Galapagos Islands.

265. OCIMUM MICRANTHUM Willd. Enum. 630. 1809. A fragrant annual with minute blue flowers, in plantation clearings, Manglaralto (11,437). In Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 161. 1925, the "wild basil" is said to extend from Florida and the West Indies to continental tropical America.

SOLANACEAE

266. BRACHISTUS **Haughtii** sp. nov.—Frutex parvus, 5 dm. altus, ramis (4–5 mm. diametro) teretibus vel paullo angulatis cortice rimoso grisea vel erubescente, ramulis novis strictis flavo-pubescentibus; foliis alternis vel in ramulis brevibus congestis, ovatis, integris, acutis vel obtusis, basi attenuatis (interdum cum petiolo 2 mm. longo) utrinque in nervis et margine sparse flavo-pubescentibus, 10–25 mm. longis, 4–9 mm. latis; floribus axillaribus solitaribus pedunculatis; calyce pubescenti paullo 5-angulato, 3–4 mm. lato, 5 dentibus retrorsis supra angulis calycinis; corolla rotata, plicata, lutea, 1 cm. diam. limbo 5-dentato; staminibus limbo inclusis; stylo incrassato stigmate capitato; bacca orbiculari 4–5 mm. diametro, viridi, 7-seminata, seminibus flavo-brunneis.

Forming little thickets on the grassy summit of Cerro Prieto, 2500 ft., Amotape Hills, Piura, Peru (H&S 11,621, type in hb. Brooklyn Bot. Gard.) (Pl. 19, fig. 2). This plant is quite distinct from *B. pubescens* Stewart of the Galapagos Islands, which is a soft dichotomously-branched, very pubescent plant. The relationship appears to be much closer to *B. Hookeriana* Miers, Ann. & Mag. Nat. Hist. ser. 2, 3: 262. 1849, described from Jameson's collection from Cerro Santa Ana, Guayaquil; but that species is described as having leaves approximately 7 cm. long and 4 cm. wide, far exceeding the dimensions of the small-leaved *B. Haughtii.* The peculiar calyx-teeth are much the same as in the Mexican *B. diversifolius* Miers, as represented by *Pringle* 6505 from Morelos. Mr. Haught has previously collected the plant on Cerro Prieto, but never with flowers.

267. BROWALLIA ELATA L. Sp. Pl. ed. 2, 880. 1763; Curtis, Bot. Mag. t. 34. 1787. This is one of the few colorful plants of the coastal region of Ecuador and Peru, where it comes into flower rather late in the season. It is locally abundant in wet seepages and in the shade of thickets in the hills east of Talara (H&S 11,555) where it frequently grows with *Spilanthes alba.* I also collected it on the Puntilla at Salinas (11,242). When well developed it is a much branched herb 1–1½ ft. high with pale blue flowers (1.5 cm. diam.) frequently shading into dark blue. The calyx is elongate (8–10 mm. long), including the narrow acuminate lobes, which remain erect even in fruit, and moderately covered with appressed, somewhat incurved strigose pubescence. The peduncles are 1–5-flowered, as in the original Linnaean description, and the corolla-tube short-glandular. The specimens collected agree very well with t. 34 of Curtis. How such a plant could have been considered by Bentham (in DC. Prod. 10: 197. 1846) as a synonym of the soft-leaved and soft-stemmed *B. demissa,* with its flowers single, is hard to understand. *B. elata* came originally from Peru, but the collector seems to be unknown, and Linnaeus obtained the seeds from Philip Miller. It is closely related to *B. grandiflora* which has larger flowers, less-appressed and rougher pubescence on the calyx, and with broad, obtuse, and divergent calyx-lobes. It was collected, along with *Ruellia floribunda,* by Cruckshanks in 1839 in the Canta Valley between Lima and Pasco, Peru. The absurd story of the naming of three *Browallia* species, *B. demissa, B. elata,* and *B. alienata* by Linnaeus in respect to a quarrel with Browallius, is still current, though it was well disposed of by Richter, Cod. Linn. 610. 1840. *B. alienata* was known to Linnaeus only through Miller's figure, and was supposed also to have come from Peru; by Index Kewensis it is considered as a synonym of *Ruellia paniculata.* Additional collections of *Browallia elata:* PERU: *Horton* 11,585 from La Brea and *Weberbauer* 5942. ECUADOR: Oil Camp between Salinas and Guayaquil, *Hitchcock* 20,469; Caraques, *Anthony & Tate,* June, 1923.

268. CACABUS PROSTRATUS (Dombey) Bernh. in Linnaea 13: 360. 1839. *Physalis prostrata* Dombey ex Lam. Encyc. 2: 102. 1786; Ill. 2: 29. 1797 & t.

116, f. 2. 1797; Dunal in DC. Prod. 13, pt. 1: 449. 1852. *Physalis limensis* Retz, Obs. 5. 22 [1779–1791], according to Nees, Linnaea 6. 1831. *Physaloides prostrata* Moench., Meth. suppl. 178. 1802. *Thinogeton maritimum* Benth., Bot. Sulph. 142. 1845 (e desc.). *Dictyocalyx Miersii* Hook. f. Trans. Linn. Soc. 20: 203. 1847. *Thinogeton Miersii* Miers, Ann. & Mag. Nat. Hist. ser. 2, 4: 359. 1849. *?T. Hookeri* Andersson, Eugenies Resa Bot. 217. 1855. *Cacabus Miersii* Wettstein in Engler & Prantl, Pflanzenf. IV 3b: 16. 1895. *?Cacabus Hookeri* Stewart, Proc. Calif. Acad. ser. 4, 1: 137. 1911. *Cacabus multiflorus* Dammer, in Fedde, Rep. Spec. Nov. 15: 366. 1919.

Salinas, abundant in fields and waste places (11,290) (Pl. 4, fig. 2); river gravels, Quebrada Ancha, La Brea, Peru (H&S 11,623). I have seen also the following collections. GALAPAGOS ISLANDS (Gray Herbarium): Chatham I., *Snodgrass & Heller* 523; Barrington I., *S. & H.* 475; Albemarle I., *S. & H.* 167, Tagus Cove; Iguana Cove, *Stewart* 3418; Culpepper I., *S. & H.* 2; Daphne I., *Wheeler, Rose & Beebe* 85. PERU: between Piura and Nomala, 100–150 m., *Weberbauer* 5931 (G) (type coll. of *C. multiflorus*); mountains near Chosica, Lima-Oroya r.r., 1200 m., *Weberbauer* 5336; east of Talara, Prov. Piura, *Beetle* 26,200; Talara, *I.M. Johnston* 3501.

This annual of dry regions starts to flower when very small, the flowers being produced singly or in pairs in the axils of the succulent leaves. As the plant increases in size the stem becomes elongate and prostrate, the inflorescence becoming many-flowered in a paniculate manner. The flowers resemble those of a *Petunia*. They are white with a dull purplish center, marked with purple striations in the throat, and with corolla apices lightly purplish-tinged. According to Weberbauer, the plant serves as fodder in Peru. At Salinas it was locally abundant, even on the banks of salt-pits.

Specimens cited are all identical as to species, and it is surprising that Dammer made no comparison with species already described, since identical elongate inflorescences can be seen on well-developed Galapagos specimens. Riley (Kew Bull. 1925: 227. 1925) upon examination of Darwin's specimen, was unable to find any glands in the pubescence. That seems to be the situation in all specimens; the hairs are jointed and viscid and grains of quartz are frequently found adhering to them, simulating glands. The calyx increases greatly in size in conformity with the expanding capsule and at maturity the tube has ten slightly elevated ribs. The young capsule is clearly 2-celled, but at the base of the mature capsule is a stipitate, ligneous, 4-celled structure with incurving walls. Within these woody cells the lowermost seeds are attached. Mature capsules become soft (with a texture somewhat like that of *Capsicum annuum*), dark green to black, and a little elongate, with pale brown seeds buried in a dark viscid substance. The filaments are of three lengths, and are inserted at various levels on the corolla tube. The stigma is lanceolate.

In describing *Dictyocalyx Miersii*, Hooker stated that the plant was in his herbarium from the coasts of Peru and Colombia, but later botanists have erroneously treated it as endemic to the Galapagos Islands. *D. prostrata* is the type of the genus. It was grown in many European gardens from seeds sent by Dombey from Peru, and was well figured by Lamarck, Jacquin (Ic. Rar. t. 38), L'Heritier (Stirp. Nov. t. 22), and others. I have not seen an original specimen, but the corolla was described by Dunal (l.c.) as resembling a *Campanula* or *Belladonna*, violaceous, with whitened limb and radiating lines of deeper violet. By Bernhardi the stamens were stated to be of unequal length, and the calyx 10-angled, the lower part becoming larger after flowering. The plant is quite obviously the same as *D. Miersii*. The status of *C. maritimus* (Benth.) Benth.& Hooker is not very clear. It was said (Bot. Sulphur, p. 142) to have come from a sandy beach in Colombia (without further locality), but the genus has apparently not since been seen from that country. However, throughout the account in the *Sulphur*, localities in Ecuador are often noted as being in Colombia. Hinds collected also on the coast of Peru and Ecuador and the description of *Thinogeton maritimum* agrees with that of *D. Miersii*, which I suspect it to be. Bentham & Hooker reduced Miers' six species to two, differentiating the fruiting calyx of *C. prostratus* as inconspicuously 5-angled, and that of *C. maritimus* as 10-angled with 5 of the angles a little more prominent. In comparison with Bernhardi's original description of the former species this difference does not appear to be very much; it is probable that only a single species of this genus is native to the South American coast.

269. CESTRUM AURICULATUM L'Her. Stirp. Nov. fasc. 4: 71, t. 35. 1785; Francey, Candollea 6: 132. 1935; Diels, Fl. Ecuador 136. 1937. An abundant small shrub (Pl. 7, fig. 1) with fragrant greenish flowers; along stream, Manglaralto (11,453). This is primarily a Peruvian species, known from Guayas Prov. (*Orrtenoda*, without further loc., cf. Francey); and between Loja and Portovelo (*Rose* 23,332), as cited by Diels.

270. DATURA INOXIA Miller, Gard. Dict. ed. 8: no. 5, 1768. *?D. guayaquilensis* HBK. Nov. Gen. & Sp. 3: 8. 1818. Annual with white corolla 15 cm. long, on waste banks by sea, Salinas (11,066, in flower and fruit). This species, obtained by Miller from seeds originating at Vera Cruz, Mexico, has long, relatively soft spines on the fruit, and the leaves, more or less covered by soft down, are variously angled. It is characteristic of dry localities, as may be seen from citation of South American specimens in the Gray Herbarium: VENEZUELA: Margarita I., *Miller & Johnston* 83; La Guaira, *Pittier* 9845. COLOMBIA: Puerto Colombia, *Pennell* 10,242; Barranquilla, *Bro. Elias* 446; Cartagena, *Bro. Heriberto* 2082; Soplaviento, Dept. Bolivar,

Killip & Smith 14,616. PERU: Pariñas Valley, Piura, *Haught* 271; 50 mi. east of Capo Blanco, *Haught* 189. PARAGUAY: Central Paraguay, *Morong* 69. *D. guayaquilensis,* based on poor specimens, came from "locis humidis prope Guayaquil Quitensium, ad litus Oceani Pacifici. Floret Februario, Martio."

271. GRABOWSKYA BOERHAAVIAEFOLIA (L.f.) Schlecht. Linnaea 7: 72. 1832; Arnott, Linnaea 11: 485. 1837. *Lycium boerhaaviaefolium* L.f. Suppl. 150. 1781. *Lycium heterophyllum* Murr. Comm. Goett. 6: 8, t. 2. 1785. *Ehretia halimifolia* L'Herit. Stirp. 1: 45, t. 23. 1786. ?*Lycium geniculatum* sensu Stewart, Proc. Calif. Acad. ser. 4, 1: 138. 1911.

A glaucous-leaved, orange-berried shrub with arching branches on sandy cliffs at the seashore, Negritos, Piura, Peru (H&S 11,609). This was collected in the same locality by Haught and is the same as *Johnston* 3506 from Talara. The immature fruit is also identical with my collection no. 41 from Indefatigable Island in the Galapagos (cf. Amer. Jour. Bot. 22: 254. 1935). The species is known also from Duncan, Hood, and Seymour Islands.

This shrub was grown at Paris from seeds sent by Joseph Jussieu from Peru. According to Lamarck, Encyc. 3: 510. 1791, the flowers are white or clear violet, pedicelled, odorous, in short panicles and corymbiform, and the calyx somewhat truncate with five sharp teeth; according to L'Heritier the yellow bilocular fruit is two-seeded, the seeds with three teeth at the base. All these characteristics agree very well with the shrub of the Peruvian coast. This type of fruit characteristic of *Grabowskya* has been considered by Bitter in Engler, Bot. Jahrb. 54, Biebl. 119: 17. 1916, as a drupe rather than a berry, because of the stony concretion surrounding the two seeds, except at the base. Dammer in Engler, Bot. Jahrb. 50, Biebl. 111: 52. 1913, has reported *Weberbauer* 3783 from 400 m. alt. at Cajamarca, Peru, as the first definite example found outside of cultivation. Judging from the endemic character of the coastal flora, it is easy to see how this species, along with *Zinnia peruviana* (of which the seeds were also collected by Jussieu) could remain unknown in their native state for such a long time. Schlechtendal (l.c.) mentions that the plant should be placed in *Lycium* rather than in *Ehretia* (Boraginaceae) "despite the Heritier plate and description, in which no botanists seem to have had any faith."

272. LYCIUM TWEEDIANUM Griseb. Pl. Lorent. 168. 1874; C. L. Hitchcock, Ann. Missouri Bot. Gard. 19: 221. 1932. Erect, or frequently pendent from the summit of sea cliffs, Puntilla, Salinas (11,133); Punta Ayangue (11,358); Punta Centinela (11,402); climbing over bushes in fields, La Libertad (11,304).

The flowers (coll. Feb. 15th) are pale green and disappear quickly, and I could find none after this date. They are followed by bright red, ovate berries. The flower dimensions are practically identical with those given by Hitchcock for *L. minimum* of the Galapagos Islands (l.c.) of which only a single flower is known. In view of the similarity of the flora of the Galapagos Islands and the Ecuadorean coast, it is quite probable that the island plant is merely a form in which the leaves tend to be reduced in width. The leaves of the Ecuadorean shrub may become as much as 5 mm. wide; in *L. minimum,* as described by Hitchcock, they are not more than 1.25 mm. wide. Collections of *Lycium* from the coast of Ecuador and Peru have been referred (see *Hitchcock,* p. 223) to *L. Tweedianum* (a widespread species described originally from the interior of Argentina) with a corolla-tube 4.5–6.5 mm. long, and leaves 2–15 mm. broad. Previously the *Lycium* of this coastal area had been treated as *L. salsum* Ruiz & Pavon, which came from Lurin, Peru, just south of Lima, according to Poiret (Encyc. Suppl. 3: 428. 1814), but the corolla was described as pale reddish and its lobes viscous, elongate, and reflexed; the berry elongated and pointed. These characters do not seem to apply to our plant which has green flowers and rounded obtuse fruit.

The following measurements of flowers will serve for correlation:

L. Tweedianum (description by Hitchcock): calxy 2.5–4 mm. long; ⅔ as wide; corolla lobes 3–3.5 mm. long [key p. 206: corolla lobes 2–3 times as long as broad]; corolla tube 4.5–6.5 mm. long.

No. 11,133: pedicel 6–7 mm. long; calyx 2–2.5 mm. long, 2 mm. wide at apex, reticulate, glabrous except the ciliate-puberulous lobe-apices; corolla lobes 2.5 × 1.5 mm. with anthers slightly exserted, 1 mm. long; corolla tube 2.5 mm. long; 1.5 mm. wide.

L. minimum (description by Hitchcock): calyx 2–3 mm. long, 1.5–2 mm. wide at apex; corolla lobes 2–2.5 mm. long, ⅔ as long as tube; corolla tube 3–3.5 mm. long, 2 mm. wide.

L. Tweedianum is the most widely-distributed species of *Lycium* and has a geographic distribution similar to that of *Kallstroemia adscendens,* which likewise appears in western Argentina. In Ecuador it has been collected several times in the vicinity of Guayaquil, also by Spruce at Chanduy (6505), by A.S. Hitchcock near Machala (21,111), and by Eggers at El Recreo (14,964) (cf. C.L. Hitchcock, p. 223). Andersson (p. 131) got it (as *L. salsum* R.&P.) at Puná Island. Of all the specimens which I have examined the flowering branch collected by me on the Puntilla at Salinas is most like *Miller & Johnston* 153 from Margarita Island.

273. PHYSALIS ANGULATA L. Sp. Pl. 183. 1753. Glabrous annual with small yellow flowers and angled inflated pods, low ground north of Ancon (11,432); river gravels of stream at west base of Chanduy Hills (11,062); rocky banks, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,547). This weed, widespread in both hemispheres, is also known from several islands in the Galapagos.

274. SOLANUM **amotapense** sp. nov.—Frutex inermis 1 m. altus, ramis contortis (5–6 mm. diametro) glabris, cortice sulcato griseo, ramulis novis pedunculisque brevissimis pilis simplicibus (rarius


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bifurcatis vel ramosis) dense obtectis; foliis alternis, congestis, laminis tenuibus ovato-acuminatis, cordatis, usque ad 9 cm. longis, 5 cm. latis, supra in nerviis, subtus in laminis sparse pubescentibus; petiolis 2–4 cm. longis; inflorescentia terminali, plerumque racemosa, pedunculis 4–5 cm. longis; calyce 3–4 mm. lato, urceolata, parte inferiore gibbosa, margine 5-dentato, dentis 2 mm. longis, strigosis; corollis albis, breve campanulatis, 1–1.5 mm. diam., limbo 5-lobato, lobis acutis, extus pubescentibus; filamentis 5, aequalibus, basi tubo adnatis, 0.5–1 mm. longis; antheris oblongis, 5–6 mm. longis, 1.5–2.0 mm. latis, apice angustis, poris apicalibus; style angusto, glabro, 9 mm. longo; baccis globosis, glabris, brunneis, 1–1.5 cm. diam.; seminibus deltoideis, 5 mm. diam. valde compressis, flavis.

Spreading bushes near the summit of Cerro Prieto, 2300 ft. alt., Amotape Hills, Piura, Peru (H&S 11,634, type in hb. Brooklyn Bot. Gard.) (Pl. 14, fig. 1). This is the same clump represented by *Haught* 195 (US), in flower; it has a superficial resemblance to *S. alboluteum* Persoon.

275. S. NIGRUM L. Sp. Pl. 186. 1753. Waste ground by the sea, Salinas (11,070). This is the weed with thin glabrous leaves and short anthers (1.5 mm. long) common in temperate and tropical regions.

276. S. CYATHOCALYX Van Heurck & Muell.-Arg. Obs. Bot. 65. 1870; Morton, Contrib. U.S. Nat. Herb. 29: 57. 1844. *Lycianthes cyathocalyx* Bitter, Abh. Naturw. Ver. Bremen 24: 400. 1919.

An abundant white-flowered subscandent shrub in which the terminal flowering portions of the branches constantly elongate, so that the fruits are normally left behind to mature (usually singly) in the angles of the branches. The flowers are slightly zygomorphic, as may be observed in the filaments (two are only 1 mm. long) and the upcurved style. Ridge north of Santa Elena (11,366) (Pl. 19, fig. 4); Punta Centinela (11,411); Punta Ayangue (11,357); low ground between Ancon and Santa Elena (11,169); summit of Chanduy Hills, 1800 ft. (11,034); granitic rocks, Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,528). The cuneiform seeds are wedged together to form a hard core, the seeds themselves being of a stony texture due to a surface layer of vertically elongated sclerotic cells. The species has been previously known only from *Spruce* 6501 from Chanduy.

277. SOLANUM **talarense** sp. nov.—Suffruticosum, caule ca. 0.5 m. longo, procumbente vel suberecto, ramis paucis (5 mm. diam.) flavis, dense aculeatis et pubescentibus; foliis alternis (internodis 1–3 cm. longis), laminis tenuibus, flavescentibus, ovatis, acutis, basi subtruncatis vel in petiolem irregulariter lateribus desinantibus, 3–8 cm. longis, 1–5 cm. latis, utrinque pubescentibus cum pilis sessilibus stellatis, pilis simplicibus septatis et glandulis stipitatis; petiolis 0.5–3 cm. longis, parum aculeatis; inflorescente terminali, pedunculis paucis, plerumque bifloris; calyce profunde 5-loba, aculeata; corollis viridi-flavis, 3 cm. diam., profunde (usque ad basim) 5-lobis, lobis 3–4 mm. latis, acuminatis, extus stellato-pubescentibus; filamentis 5, glabris, 1–1.5 mm. longis, hyalino-alatis; antheris linearibus, apice angustis, rimis terminalibus anticis hiantibus, dein lateralibus dehiscentibus; stylo filiforme, 9 mm. longo, parte inferiore glanduloso, stigmate parum incrassata; fructo immaturo glanduloso.

Procumbent on dry gravels, Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,534, type in hb. Brooklyn Bot. Gard.) (Pl. 19, fig. 3). This plant, with dull greenish-yellow flowers, was previously collected (in flower) at Talara, *Haught* 72 (US). Its closest resemblance is apparently to the purple-flowered *S. crinitipes* of the Ecuadorean Andes, but is entirely different in the thin leaves, color of flowers, and type of pubescence. The two species of *Solanum*, which I have described as new, I have not found named in the extensive collections of the Gray Herbarium, U. S. National Herbarium, or the New York Botanical Garden, and I have not seen descriptions in recent papers by Bitter or by Werdermann to fit these plants.

SCROPHULARIACEAE

278. CAPRARIA PERUVIANA Benth. in DC. Prod. 10: 430. 1846. Low bushes along stream-bed between Ancon and Santa Elena (11,185); bushes 3 ft. high at edge of salt marsh, Punta Ayangue (11,337); in wet quartz sand, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,524). This species is not far removed from the widely-distributed *C. biflora* L., but differs in its entirely glabrous character and the lanceolate acute leaves. None of the material which I collected was in flower, but all heavily fruiting. It was figured by Feuillée (1714) and known by him and by Linnaeus as *Capraria peruviana, agerati foliis absque pediculis,* but no binominal appears to have been formed until the time of Bentham, if we may overlook that of *Witheringia? salicifolia* Hooker, Bot. Miscell. 2: 231. 1831, based on a collection by Cruckshanks from Lurin, near Lima. *C. peruviana* extends along the coast from Lima to Ecuador, and is occasional in the Galapagos Islands.

279. GALVESIA BALLII Munz, Proc. Calif. Acad. Sci. 15: 379. 1926. A shrub with large scarlet flowers on the dunes at Negritos, Piura, Peru (H&S 11,610) (det. Pennell). Endemic to western Peru. I saw no sign of this species on the Ecuadorean coast, but Stewart (p. 141) has doubtfully referred plants collected by him on Jervis Island in the Galapagos to *G. fruticosa.*

280. SCOPARIA DULCIS L. Sp. Pl. 116. 1753;

Plate 19.—Fig. 1. *Solanum amotapense* (*H&S* 11,634, Peru) flower × 1.5; anther and seed × 2.—Fig. 2. *Brachistus Haughtii* (*H&S* 11,621, Peru) expanded corolla, style, anther, calyx, fruit, and seed, all × 2.—Fig. 3. *Solanum talarense* (*H&S* 11,534, Peru) flower and calyx × 1.5; anther × 2; young fruit and individual types of pubescence much enlarged.—Fig. 4. *Solanum cyathocalyx* (11,366, Ecuador) corolla × 1.5; calyx, style, stamen, and seed, all × 2.

Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 181. 1925. A much-branched slightly shrubby plant with small pale violet flowers. Low grounds between Ancon and Santa Elena (11,178); river gravels, Quebrada Mogollon, Amotape Hills, Peru (11,546). One of the most widespread of weeds in the tropics of both hemispheres; known from the Galapagos Islands.

281. STEMODIA aff. DURANTIFOLIA (L.) Sw. Obs. 240. 1791; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 184. 1925. *S. Berteroana* Benth. in DC. Prod. 10: 384. 1846. *S. erecta* (Browne) Minod, Bull. Soc. Bot. Genève ser. 2, 10: 212. 1918 (nomen illegitimum).

Decumbent plant with small white flowers, in river gravels, Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,542). *S. durantifolia* is a weedy plant extending from California and the West Indies southward to Chile and Paraguay; it has not been reported from the Galapagos Islands.

MARTYNIACEAE

282. PROBOSCIDIA PERUVIANA Van Eseltine, N.Y. Agric. Expt. Sta. (Geneva) Technical Bull. 149: 17, fig. 6. 1929. Sandy places east of Negritos, Piura, Peru (H&S 11,581). The species was described from a single collection, *Haught* 14 from Talara. The leaves in no. 11,581, are frequently deeply 3-lobed, with blades as much as 4 dm. long and 5–6 cm. wide. These dimensions are considerably greater than given by Van Eseltine. The fruit has long horns (about 10 cm.) which are twice as long as the body of the fruit.

ACANTHACEAE

283. ELYTRARIA IMBRICATA (Vahl) Pers. Syn. 1: 23. 1805. *Justicia imbricata* Vahl, Eclog. 1: 1, t. 1. 1796. Low, often much-branched perennial, with small flowers obscured by scales of the branches, Puntilla, Salinas (11,114); Chanduy Hills, 1200 ft. (11,035). Widely distributed in tropical America.

284. JUSTICIA CARTHAGINENSIS Jacq. Enum. 11. 1760; Stirp. Amer. 5, t. 5. 1763; Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 216. 1925. Herb 2 ft. high with divaricate branching stems and purple flowers, Chanduy Hills, 1200 ft. (11,044). Widespread from the Bahamas southward. The original collection came from Carthagena, Colombia.

285. J. COMATA (L.) Lam. Encyc. 1: 632. 1785. *Dianthera comata* L. Syst. ed. 10. 850. 1759. *Stethoma comata* Britton in Britton & Wilson, Sci. Surv. Porto Rico & Virgin Isls. 6: 218. 1925. Herbaceous plant with panicles of minute white flowers, only about 3 mm. long. Moist plantation clearings, Manglaralto (11,502). The leaves (reaching 7 × 3.5 mm.) are broader than usual in the species, and the capsules (4 mm. long) are beset with short trichomes even at maturity. *J. comata* was collected by Haenke and by Hartweg at Guayaquil (cf. Nees in DC. Prod. 11: 381. 1847). A glabrous variety of *Leptostachya Martiana* Nees was noted from Guayaquil by Bentham (Bot. Sulphur, p. 147) but is probably not this plant. *J. comata* is found in wet grounds at low elevation from Cuba to South America.

286. RUELLIA FLORIBUNDA Hook. Bot. Miscell. 2: 236. 1831. *Aphragmia rotundifolia* Nees in Benth. Bot. Sulphur 146. 1849. *Dipteracanthus floribundus* Nees in DC. Prod. 11: 141. 1847. *D. viscidus* Nees in DC. Prod. 11: 141. 1847.

Below top of sea-cliffs exposed to salt spray, Puntilla, Salinas (11,129). The circular seeds (2–2.5 mm. wide) are covered with long white viscid pubescence. I have seen specimens ranging from central Bolivia and Peru to Guayaquil and the Galapagos Islands, as follows: BOLIVIA: Yungas, 6000 ft., *Rusby* 2410 (NY). PERU: near Corcona, vic. Lima, Rose 18,677 (NY) [this collection is perhaps closest to the description of *R. floribunda*, which came from the Valley of Canta, some fifty miles northeast of Lima (coll. *Cruckshanks*)]; Talara, Dept. Piura, *Haught* 36 (US, NY). ECUADOR: Guayaquil, *Hartweg* 688 (NY), cited by Bentham in *Pl. Hartweg* 122. 1839, as *Aphragmia Haenkei* Nees ? [specimen large-leaved, almost exactly as in 11,129]; *Gaudichaud*, photograph (G); *Holway* 800 (NY); *A.S. Hitchcock* 19,961 (NY); vic. Duran, *Rose & Rose* 23,591 (NY). Chatham I., Galapagos, *Schimpff* 182 (B). Nees (in DC. Prod. l.c.) cites *D. viscidus* from Chile (hb. *Haenke*).

The leaves vary from rotund (often with blades only 2 cm. long) to ovate (with blades as much as 7 cm. long). *R. floribunda* was well described, and undoubtedly is the same as *Dipteracanthus viscidus*. Specimens are frequently named *R. paniculata* or *R. albicaulis*. The genus *Aphragmia* Nees was characterized by capsules greatly narrowed into the sterile lower half—the seeds therefore only 2 or 4, in contrast to the many seeds of *Ruellia paniculata*, which has an unconstricted capsule. In 1847 Nees included *Aphragmia*, comprising *A. rotundifolia* and *A. Haenkei*, under *Dipteracanthus*, the name *Ruellia* being restricted to Old World species. *R. floribunda* has firm, rather blunt leaves, a diffuse inflorescence, broad calyx lobes with discoid glands, corolla lobes erect and the tube expanded, the anthers 3–4 mm. long; *R. inundata* (*R. albicaulis*)[14] has flaccid acute leaves, a rather dense axillary inflorescence due in part to the prominent oblong bracts, linear calyx lobes with small glands, corolla lobes spreading with tube nearly straight, and anthers 2–2.5 mm. long.

287. RUELLIA **pacifica** sp. nov.—Herba 3 dm. alta, ramis suberectis pilis brevibus incurvis obtectis; laminis ovatis, acutis, integris, 1.5–8 cm. longis,

[14] *R. inundata* HBK. Nov. Gen. & Sp. 2: 239. 1818; Leonard, Bot. Maya Area [Carnegie Publ. 461] 208, fig. 10. 1936. *R. albicaulis* Bertero ex Spreng. Syst. 2: 822. 1825. *Aphragmia Haenkei* Nees in Lindley, Nat. Syst. ed. 2, p. 444. 1836. Kunth's collection came from Colombia, as did Bertero's (from the Magdalena River). The species is known from Mexico to Colombia, the following being representative: MEXICO: Oaxaca, Pringle 6259 (B). COLOMBIA: Santa Marta, *H. H. Smith* 1410 (NY); Dept. Atlantico, *Elias* in 1932 (NY); Dept. Bolivar, *Pennell* 4028 (NY). A number of specimens are cited by Leonard from Yucatan.

1–3 cm. latis, supra adpresso-strigosis, subtus dense pubescentibus; petiolis 1–5 mm. longis, ciliatis; floribus axillaribus; calycis laciniis 10–15 mm. longis, hispidis, anguste lineari-lanceolatis; corollis purpurascentibus, plerumque 5 cm. longis, extus pubescentibus, lobis 11 mm. latis; capsulis 10 mm. longis, 5 mm. latis, puberulis, 4–6-spermis; antheris 4 mm. longis, pubescentibus, non-muticis.

Rough herb a foot high with large purple flowers, dry fields, Baños San Vicente, Guayas, Ecuador (11,500, type in hb. Brooklyn Bot. Gard.); smaller-flowered plant, Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,527) Pl. 20, fig. 4). This plant seems to be restricted to the dry area of southwest Ecuador and northern Peru. In the large collection at the Gray Herbarium I have seen only two additional specimens: *Hitchcock* 19,964, from cut-over land between Guayaquil and Salinas; *H. E. Stork* 11,378, Huancabamba, Piura, Peru. The species is closely allied to *R. geminiflora,* from which it differs in much larger flowers, larger leaves, pubescent seeds and anthers (cf. description by Leonard, Bot. Maya Area [Carnegie Publ. no. 461]: 205. 1936). A specimen of *R. geminiflora* from Venezuela is illustrated (Pl. 20, fig. 3). This conforms well with Kunth's description of the species which came from the vicinity of Ibaque, Colombia, and is the narrow-leaved plant characteristic of Brazil and Argentina. *R. pacifica* is probably the plant collected by Andersson on Puná Island, noted by Areschoug (Bot. Eugenie III, p. 131) as *"Calophanes ovatus?* Benth. Pl. Hartw. p. 89" and differing from the *Hartweg* Collection [from Sumpanga Mt., Guatemala] in having fewer flowers with broader and more pilose leaves. By Nees, in DC. Prod., 11: 108. 1847, *Calophanes ovatus* is said to be "persimilis *C. oblongifolia"* which is a small-flowered and narrow-leaved plant of the coastal plain of Georgia and Florida. Of all the specimens seen, *R. pacifica* is closest to *Pringle* 4438 (sub *R. geminiflora*), from Jalisco, Mexico, from which it differs materially in the densely pubescent lower-leaf surface and pubescent anthers. It has, however, the same minute cup-shaped glands scattered throughout the inflorescence.

288. TETRAMERIUM NERVOSUM Nees in Bentham, Bot. Sulphur 148, t. 48. 1846. A slender plant with prominently-nerved bracts, past flowering, among shaded rocks, northwest slope of ridge, Santa Elena (11,372). Known previously from Puná Island only.

RUBIACEAE

289. BORRERIA ANDERSSONII Standley, Field Mus. Bot. 7: 245. 1931. Annual species resembling the widespread *B. laevis,* but with capsules twice as large (4.5–5 mm. long), and with larger flowering heads. Lower slopes of Chanduy Hills, 300 ft. (11,052). The species, confined to this vicinity, has been known from three previous collections: Puná Island (*Andersson*); between Guayaquil and Salinas (*Hitchcock*); near Guayaquil (*Mille*).

290. B. LAEVIS (Lam.) Griseb. Goett. Abh. 7: 231. 1857; Standley in Macbride, Fl. Peru, pt. 6: 251. 1936. *Spermacoce laevis* Lam. Ill. 1: 273. 1791. Manglaralto (11,488). Widely distributed in tropical America. Known from the Galapagos Islands.

291. CHIOCOCCA ALBA (L.) Hitchcock, Rept. Missouri Bot. Gard. 4: 94. 1893; Standley in Macbride, Fl. Peru, pt. 6: 123. 1936. *Lonicera alba* L. Sp. Pl. 175. 1753. Somewhat climbing shrub with shiny leaves and yellow flowers, banks of stream, Manglaralto (11,452). Widespread in tropical America from Florida southward. In the Galapagos Islands it appears to be confined to the moister areas. I saw nothing of it in the arid parts of Ecuador, and Standley, Field Mus. Bot. 7: 217. 1931, has cited it only from El Recreo (*Eggers* 15,409).

292. DIODIA INCANA Areschoug, Eugenies Resa Bot. III: 126. 1910. Erect branched or unbranched annual with pale blue flowers, rocky soil, Puntilla, Salinas (11,088, 11,244); dry hills north of Punta Carnera (11,307). This is perhaps only a variation of *Diodia teres* Walt. closest to var. *setifera* Fernald & Griscom, Rhodora 39: 307. 1937. Areschoug recognized its affinity to *D. teres,* from which he distinguished *D. incana* by the tetragonous culm, and very scabrous leaves almost silvery below. *D. teres* is cited by Standley in Macbride, Fl. Peru, pt. 6: 247. 1936, from Serrán, southeast of Piura (coll. *Weberbauer*). It ranges northward to Rhode Island (United States).

293. OLDENLANDIA CORYMBOSA L. Minute scabrous erect annual only 2–3 cm. high, with white to pale blue flowers, dunes, Salinas (11,190). The corollas are only 1 mm. long; the capsules 2 mm. wide and 1 mm. high. A very dwarfed form, with solitary flowers only, of this widespread plant.

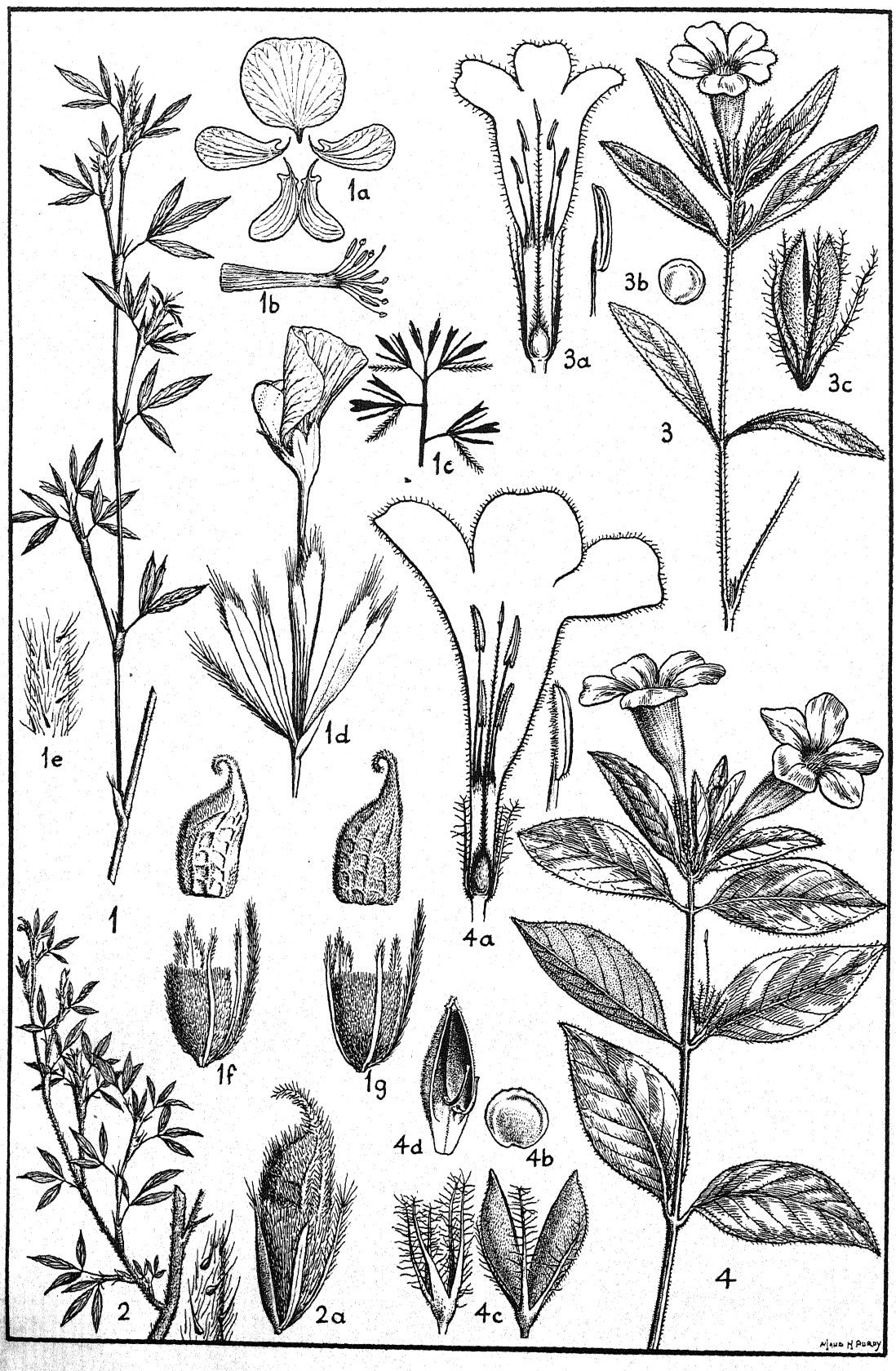
294. PSYCHOTRIA ALBA R.&P. Fl. Peruv. 2: 58, t. 205, f.a. 1799; Standley in Macbride, Fl. Peru, pt. 6: 182. 1936. Shrub 8 ft. high with reddish leaves, in forest, Manglaralto (11,451). A species widely distributed in South America.

295. SPERMACOCE CONFUSA Rendle, Jour. Bot. 74: 12, with fig. D–F. 1936. *S. tenuior* Gaertn. De Fructibus, t. 25. 1788; not L. Annual with verticils of minute bluish-white flowers, low ground north of Ancon (11,434). Florida, West Indies, and Central America to equatorial South America. This is *S. tenuior* of Grisebach and other authors. *S. tenuior* L., as Rendle has shown (Jour. Bot. 72: 329. 1934), is *S. riparia* Cham. & Schlecht.

296. SPHINCTANTHUS sp. Bush or small tree (8 ft. high) with large bright orange flowers as much as 6 cm. diameter (det. Standley). Around pools, west base of Chanduy Hills (11,029). A *Sphinctanthus* was reported by Weberbauer, in Engler, Bot. Jahrb. 63: 39. 1929, from Tumbez, Peru.

CUCURBITACEAE

297. APODANTHERA BIFLORA Cogn. Diagn. Cucurb. 2: 43. 1877; Cogn. in Engler, Pflanzenr. IV. 275: 63, fig. 18. 1916; Macbride, Fl. Peru, pt. 6:


1a
1b
1c
1d
1e
1f
1g
1
2
2a
3a
3b
3c
3
4a
4b
4c
4d
4
MAUD H. PURDY

358. 1937. A dioecious herbaceous vine, abundant in the dry region of southwestern Ecuador and one of the earliest plants to bloom after the rains. By the middle of February, in 1941, banks and open woods were enlivened with its large, pale yellow flowers. Collections were made on the ridge at Santa Elena, February 22 (11,107), and in nearly mature fruit north of Punta Carnera, March 7, 1941 (11, 296). It was also seen, after rains, in the vicinity of Talara, Peru, and was collected by Haught at Negritos (no. 46) and by Weberbauer (no. 5944) near Piura. This plant, known as "yucca del monte," has a root which is edible after cooking. The ripe fruit is about 6 cm. long. Leaf-blades, usually cordate, are either simple or trilobed, sometimes incised to the base. The corolla lobes, which are acuminate and bristle-tipped, reach 3 cm. in length; they are much too short and blunt in Cogniaux's illustration. The original collection of this species was made by Spruce at Chanduy.

298. CUCUMIS DIPSACEUS Ehrenb. in Spach, Hist. Nat. Phan. 6: 211. 1838; Macbride, Fl. Peru, pt. 6: 382. 1937. A vine with small yellow flowers, the leaves and stem covered with irritating prickles, on sandy roadsides and along the beach, Salinas (11,384A, 11,420, & 11,423); river gravels, near La Brea, Peru (H&S 11,565) together with a form with incised leaves and smooth fruit (H&S 11,643). A species introduced from Africa, collected by Haught (no. 106) at Talara.

299. CYCLANTHERA LEPTOSTACHYA Benth. Bot. Sulphur 99. 1845, e descr. Climbing over low bushes, Puntilla, Salinas (11,103 & 11,076); Punta Centinela (11,409); river gravels, near La Brea, Piura, Peru (H&S 11,592). The original collection from Salango, Ecuador, lacked the pistillate flowers. It was described as resembling *C. pedata,* but with much more slender branches and with smaller leaves. These collections, with the exception of that from La Brea, came from within 20 miles of the type locality. All conform to Bentham's description except for shorter peduncles and larger number of seeds. Perhaps *C. leptostachya* represents a much-reduced *C. pedata,* with terminal leaf segments only 3.5–6 cm. long and 1–1.5 cm. wide.

300. ELATERIUM CARTHAGENENSE Jacq., Enum. Pl. Carib. 31. 1760; Macbride, Fl. Peru, pt. 6: 367. 1937. Damp woods, slope of Chanduy Hills, 1200 ft. (11,038); Manglaralto (11,461). The leaves are unusually large, and as in var. *cordatum* of the Galapagos Islands, they show no lobation.

301. LUFFA ASTORI Svenson, Amer. Jour. Bot. 22: 256. 1935. An abundant high-climbing gourd, with fruit of the preceding year conspicuous. Ridge north of Santa Elena (11,099); Puntilla, Salinas (11,112 & 11,223); low ground between Ancon and Santa Elena (11,175); Quebrada Ancha near La Brea, Peru (H&S 11,615) (Pl. 3, fig. 3). This species resembles *L. operculata,* but has larger flowers, the petals being 2 × 1.5 cm. Reticulation of the larger fruit is coarser, and leaves are broader. It is undoubtedly *L. operculata* var. *lobata* Cogn. in Mart. Fl. Bras. 6, pt. 4: 13. 1878, described from the Daule River at Guayaquil (coll. *Gaudichaud-Beaupré*). *L. Astori* was described from the Galapagos Islands, and is otherwise known only from southwestern Ecuador and northwestern Peru.

302. MOMORDICA CHARANTIA L. Sp. Pl. 1009. 1753; Macbride, Fl. Peru, pt. 6: 366. 1937. On fences, Salinas (11,202). This abundant vine, widespread in the tropics of both hemispheres, has probably been introduced at Salinas, where, as Mr. Kenward tells me, it is the only green plant during the long dry season. The orange fruit splits into 3 lobes, disclosing the bright red seeds.

COMPOSITAE

303. ACANTHOSPERMUM MICROCARPUM Robinson, Proc. Amer. Acad. 38: 208, pl. 1, fig. 3. 1902; Blake, Contrib. U.S. Nat. Herb. 20: 389, p. 23, fig. f. 1921. *A. humile* DC. var. *subintegrifolium* Areschoug, Eugenie Bot. III: 125. 1910. *A. simile* and *A. Donii* Blake, Contrib. U. S. Nat. Herb. 20: 388, pl. 23, fig. d, e. 1921.

One of the most abundant weeds of the Ecuadorean coast, sometimes by the acre, around the small villages at the west base of the Chanduy Hills. Specimens with narrow leaves (1.5 cm. wide) from open headlands on the Puntilla, Salinas (11,246) are identical with my collection (no. 175) from Charles Island, the only locality from which the plant is known in the Galapagos. Sometimes the leaves are as much as 4 cm. wide, as on sandy flats at Salinas (11,151), on the Puntilla (11,316), and in sandy fields at Punta Centinela (11,394). The same plant grows on rocky banks of Quebrada Mogollon, Amotape Hills, Peru (H&S 11,545), a collection identical with *Weberbauer* 7695 from Hacienda Choza, Prov. Tumbez, and *Haught* 151 from the Mancora Valley, 50 miles w. of Tumbez (US). *A. simile* (from Guayaquil) and *A. Donii,* based on a collection of Ruiz & Pavon from Ecuador (?), Dr. Blake believes to be not distinct from *A. microcarpum. A. humile* var. *subintegrifolium,* obtained by Andersson on Puná Island, is clearly from description *A. microcarpum.* In the Galapagos Islands this plant has evidently been recently introduced.

304. ADENOSTEMMA aff. BRASILIANUM (Pers.) Cass. Dict. Sci. Nat. 25: 363. 1822. A weak erect plant with deltoid leaves (to 6.5 cm. wide) and

Plate 20.—Fig. 1. *Stylosanthes sympodialis* (11,258, Ecuador); corolla (1a) and stamens (1b) × 2.5. A diagrammatic inflorescence (1c), a single flower with associated bracts (1d), and a portion of the stem (1e) are shown much enlarged. Below, pods with joints separated: 11,258 from Ecuador (1f); *Howell* 8815 from Galapagos Islands (1g) × 5.—Fig. 2. *Stylosanthes scabra* (*Burchell* 8533A, Brazil); pod (2a) × 5, with joints not separated.—Fig. 3. *Ruellia geminiflora* (*Pittier* 7891, Venezuela); flower (3a) × 1; seed (3b) × 2; calyx and capsule (3c) × 2.—Fig. 4. *Ruellia pacifica* (11,500, Ecuador); flower (4a) × 1, seed (4b) × 2, calyx and capsule (*H&S* 11,527, Peru) (4c) × 1.5; interior of capsule (4d) × 1.5.

heads only 4–5 mm. in diameter, in plantation, Manglaralto (11,509). This is a small-flowered plant which differs from Asiatic specimens of *A. viscosum* chiefly in the wider corolla-tube and glabrous corolla lobes. Perhaps it represents the peculiar collection of *Adenostemma Swartzii* cited from Salango in Bentham, Bot. Sulphur (p. 111), "folia inferiora saepe semi-pedalia, 4–5-poll. lata."

305. BIDENS RIPARIA HBK. Nov. Gen. & Sp. 4: 185. 1820; Sherff, Field Mus. Bot. 16: 474, t. 114. 1937. Abundant on open headlands, Puntilla, Salinas (11,233); river gravels, near La Brea, Piura, Peru (H&S 11,598). Known from southern Mexico to northern Peru and central Brazil. *Andersson* 66, cited by Sherff from Puná, Argentina, I suspect came from Puná Island, Ecuador. Var. *refracta* (Brandegee) O.E. Schulz (cf. Sherff, p. 475) has dissected leaves and is abundant in the Galapagos Islands. It was collected by Haught (F139) northeast of Talara. There is little difference between such plants and "*B. Cynapiifolia* HBK." as represented by *Schimpff* 130 from San Cristobal, except for the spreading awns in Schimpff's collection.

306. BLAINVILLEA DICHOTOMA (Murr.) Cass. ex Hemsley, Biol. Cent.-Am. Bot. 4: 112. 1886. *Verbesina dichotoma* Murray, Comm. Geott. 2: 15, t. 4, 1779. *B. rhomboidea* Cass. Dict. 29: 493. 1823. Open woods on ridge, Santa Elena (11,102). A weedy plant of wide distribution, known also from the Galapagos.

307. DELILIA BIFLORA (L.) Kuntze, Rev. Gen. Pl. 1: 333. 1891; Blake, Contrib. U.S. Nat. Herb. 26: 237. 1930. *Milleria biflora* L. Sp. Pl. 919. 1753. *Elvira biflora* DC. Prod. 5: 503. 1836; Millspaugh & Chase, Field Mus. Bot. 3: 104 (with fig.). 1904. A weedy annual with two or three minute flowers hidden within the involucral bracts. Dry rocks, Punta Centinela (11,262); thickets at base of Chanduy Hills (11,005); rocky slope of Cerra Prieto, Piura, Peru (H&S 11,567A). Widely distributed in the American tropics. Though the species description of *Elvira inelegans* (Hook. f.) Rob., might apply to this plant, the genus description (under *Desmocephalum*) of a six-flowered inflorescence and a pilose pistillate corolla tube is quite different. *E. inelegans* is related to *Elvira atriplicifolia* Howell, Proc. Calif. Acad. n.s. 21: 333. 1935, described from James Island.

308. ECLIPTA ALBA (L.) Hassk. Pl. Jav. Rar. 528. 1848; S. Moore in Fawcett & Rendle, Fl. Jam. 7, pt. 5: 222, fig. 72. 1936. *Verbesina alba* L. Sp. Pl. 902. 1753. A weed, abundant and widespread in both hemispheres, growing at the margins of pools, Salinas (11,728 & 11,308); La Libertad (11,289). Known also from the Galapagos Islands. As in other regions, the achenes vary from smooth to roughened.

309. ENCELIA CANESCENS Lam. Encyc. 2: 356. 1786; Blake, Proc. Am. Acad. 49: 369. 1913 and Contrib. Gray Herb. 41: 369. 1913; Johnston, Contrib. Gray Herb. 85: 126. 1929. A bushy plant about two ft. high, abundant at Negritos, Peru (H&S 11,608) (Pl. 5, fig. 2). The yellow-rayed flowers with purplish disks are about 2 cm. in diam., and the plant is therefore one of the few colorful species of this desert region. It extends inland only a short distance. The map (fig. 20) is compiled from localities cited by Blake and Johnston. The endemic *Encelia hispida,* known from Charles, Chatham, and Barrington Islands in the Galapagos, is very closely related.

310. HETEROSPERMUM MARITIMUM HBK. Nov. Gen. & Sp. 4: 226, t. 383. 1820. A procumbent plant with rhombic-lanceolate leaves in river gravels, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,551). Some of the leaves are deeply incised (5–9 mm. wide). This plant has much the appearance of *H. rhombifolium* (Sch. Bip.) Griseb. described and illustrated by Sherff, Bot. Gaz. 76: 81, t. 8. 1923 (further discussed by him in Field Mus. Bot. 16: 642. 1937), but has narrower leaves.

311. ISOCARPHA MICROCEPHALA (DC.) Blake, Proc. Biol. Soc. Washington 39: 144. 1926 and Contrib. U.S. Nat. Herb. 26: 248. 1930. *Dunantia microcephala* DC. Prod. 5: 627. 1836. *I. divaricata* Benth. Bot. Sulphur 110, t. 41. 1845.

Border of fresh pond, Salinas (11,279). The corollas in this collection vary from purple to white. The species is known only from the region of the Gulf of Guayaquil, having been described from Puná Island. It was collected by *Schimpff* at Guayaquil (no. 223) and by *Eggers* at Balao (no. 14,016) (cf. Engler, Bot. Jahrb. 28: 604. 1901). Haenke's original collection (though DC. says Mexico) probably came from Ecuador. The exterior of the corolla tube has stout barb-like hairs which point downward. They perhaps function in distributing the achene.

312. LAGASCEA MOLLIS Cav. Anal. Cient. Nat. 6: 332, t. 44. 1803; S. Moore in Fawcett & Rendle, Fl. Jam. 7, pt. 5: 208, fig. 64. 1936. A weedy annual with soft silvery pubescence, river gravels, near La Brea, Piura, Peru (H&S 11,600) and Quebrada Mogollon, Amotape Hills, Peru (H&S 11,544). This species, known from Cuba southward, does not seem to have reached the Galapagos Islands.

313. MIKANIA MICRANTHA HBK. Nov. Gen. & Sp. 4: 134. 1820; B. L. Robinson, Contrib. Gray Herb. 64: 67. 1922. Vine with cordate leaves and dull white spreading inflorescence, climbing over bushes on river bank, Manglaralto (11,477). The typical form of this widespread species. The collection by *Eggers* (14,336) at Balao, and treated by Dr. Robinson as var. *cynanchifolia,* is later referred by him to a pubescent and small-headed form of *M. congesta* DC. (cf. Contrib. Gray Herb. 104: 62. 1934).

314. ONOSERIS aff. INTEGRIFOLIA Lessing, Linnaea 30: 343. 1830. A pink-rayed, apparently annual, species on the summit of Cerro Prieto, 2500 ft., Piura, Peru (H&S 11,618). The spine-tipped involucral bracts are closely appressed, and the leaves vary from 0.5–2 cm. wide. The type was collected in Peru by Dombey. This plant is also very

close to, and perhaps identical with *O. adpressum* (Hook.) Lessing, Syn. 183, represented by t. 3115 in the Botanical Magazine, though the rays (ca. 15) in no. 11,618 are about twice as many. *O. adpressum* was obtained by Cruckshanks from Peru, probably from the Lima-Cerro de Pasco region (cf. discussion of *Ruellia floribunda*).

315. PECTIS ARENARIA Benth., Bot. Sulphur 110. 1845; Fernald, Proc. Am. Acad. 33 [Proc. Gray Herb. n.s. no. 12]: 70. 1897. *Cheilodiscus littoralis* Triana, Ann. Sc. Nat. ser. 4, 9: 36. 1858. *Pectis Lehmanni* Hieron. Bot. Jahrb. 28: 620. 1901.

A perennial with thick prostrate branches which often reach a meter in length, making the plant an effective sand-binder. The flowers are large for the genus, the heads with expanded rays being as much as 2 cm. across. It is abundant on the dunes at Salinas (11,200), and less abundant on the flat outer portions of the Puntilla. Bentham described the species from "sands of the sea coast, Acapulco; Conchagua; Puná, near Guayaquil," but designated no type. Other Ecuadorean collections are *Eggers* 14,811 from El Recreo, and *Lehmann* 1115 from Caraquez. The species seems to be fairly widespread on the Mexican coast (see fig. 19). *P. Lehmanni* was based on *Lehmann* 7954 from Bahia Caraquez, Ecuador. From a photograph it appears to be *P. arenaria,* and the description of a plant with prostrate stems 25 cm. long tends to confirm the identity. The pappus of *P. Lehmanni* was described as a low crown with projections reaching only ⅓ mm. in length; in this respect it is like the Salinas material (cf. Pl. 22, fig. 4) which has variable squamellae barely reaching 0.5 mm. in the disc flowers, while in the ray flowers there is frequently no trace of a pappus whatever. A similar situation is found in *Cheilodiscus littoralis,* which Triana found in coastal sands at Buenaventura, Colombia. All the South American collections seem to have a reduced pappus, whereas by Bentham the pappus of the disc flowers was described as of 10–20 very unequal scabrid bristles, 4 or 5 dilated at the base, and the others subulate. This is essentially the description given by Fernald (l.c.), who cites the longest bristles as 4 mm. in length. *P. arenaria* appears to be even more variable than *P. angustifolia* Torrey, described as having no awns or pales, but in which awns often occur (cf. Fernald, p. 60).

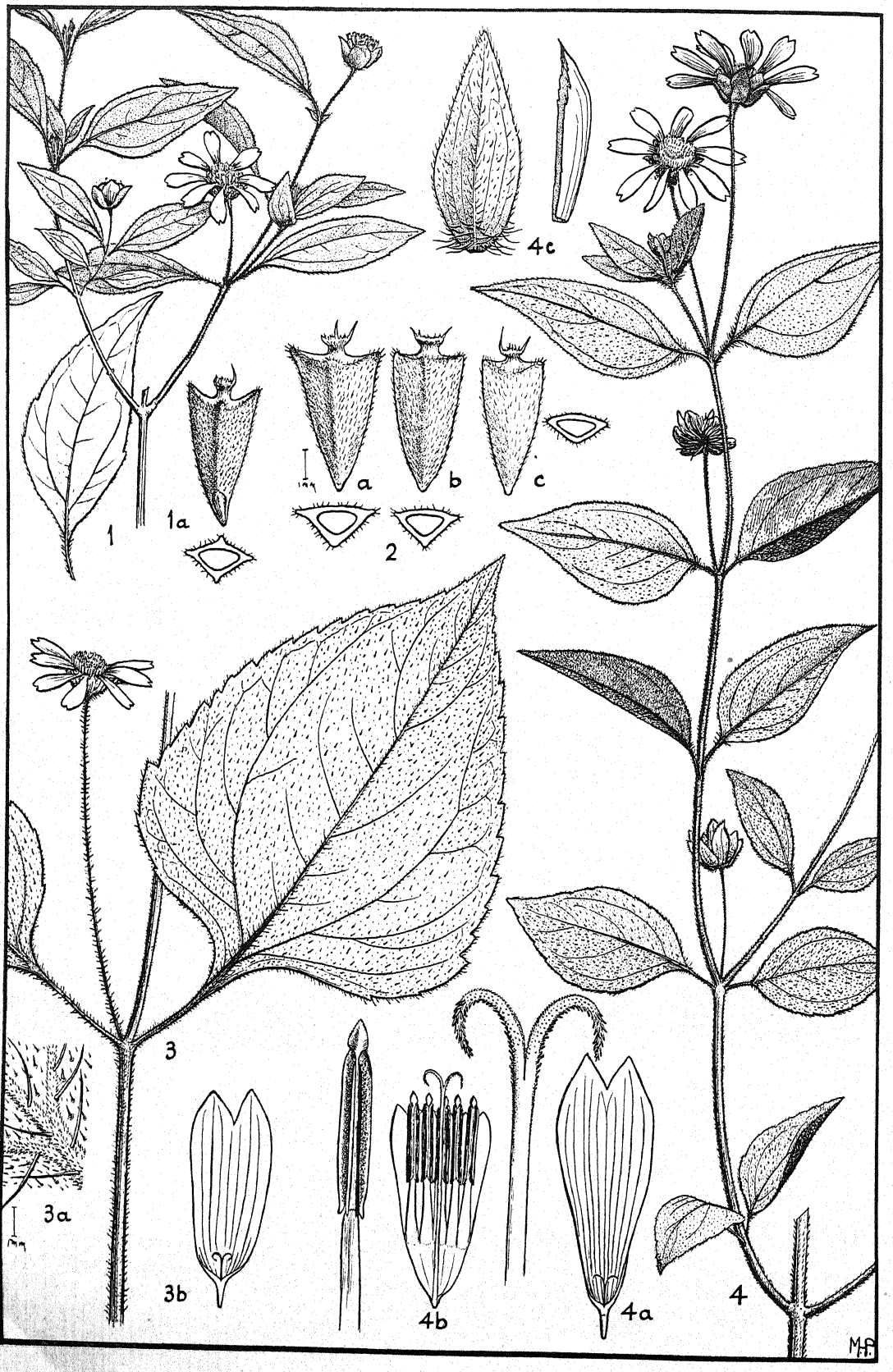
316. P. DIFFUSA Hook. & Arn. Bot. Beechey 296. 1841; Fernald, Proc. Am. Acad. [Contrib. Gray Herb. n.s. no. 12]: 78. 1897. *P. taliscana* sensu Benth. Pl. Hartw. 121. 1843 and Areschoug, Eugenies Bot. 124. 1910; not *P. taliscana* H.&A. *?P. ciliaris* L. Syst. ed. 10, 1221. 1760; Fawcett & Rendle 7, pt. 5: 264, fig. 91. 1936. *?P. prostrata* Cav. Icones 4: 12, t. 324. 1797.

Abundant on dunes and headlands on the Ecuadorean coast, Salinas (11,096, 11,078, 11,124, 11,208, 11,209); Punta Centinela (11,267); Punta Ayangue (11,345); river gravels, Quebrada Mogollon, Amotape Hills, Piura, Peru (H&S 11,520); Quebrada Ancha, near La Brea, Piura, Peru (H&S 11,583).

This exceedingly variable little plant has 5 inconspicuous yellow rays. On the dunes at Salinas it first appeared in fruiting condition on February 21, with elongate branches and pedunculate heads (cf. Pl. 22, fig. 3a); about ten days later glomerate forms appeared in the same locality with the heads sessile in leafy involucres (cf. Pl. 22, fig. 3b). Both forms remained through the growing season. At first sight these plants would seem to represent different species, but they prove to be only habital variations of the same species. On headlands of the Puntilla exposed to salt spray the congested form was more abundant, and dwarf plants only 2 cm. high were in full flower. In northwestern Peru both forms likewise grew together. Some of the plants had decumbent suffruticose stems nearly 3 mm. in diameter, and would surely be taken for perennials if seen only on herbarium sheets. Such forms were undoubtedly the basis of Bentham's report of "P. Taliscana" from Guayaquil (Pl. Hartw.), a species described from Mexico and referred by Fernald (op. cit., p. 74) to *P. capillaris.* By Bentham & Hooker, Gen. Plant. 2: 412. 1873, the Hartweg collection was cited as *P. diffusa,* known from Jalisco and Colima, Mexico, and suffrutescent at the base.

My collections might equally well be referred to *P. elongate* HBK. Nov. Gen. & Sp. 4: 262, t. 392. 1820, which questionably came from Popayan, Colombia, or to various other South American species. Finally, there is not much difference between these collections and *P. ciliaris,* of the West Indies. The elongate specimens have the same outward appearance, even as to details of the involucre, and the only difference seems to be in the pappus, which in *P. ciliaris* is composed typically of 5 paleae (basally enlarged) in the disc flowers, and 2 paleae in the ray flowers. But these are subject to variation. In my collections the bristles of the disc achenes are normally 17–21; those of the rays less. If these bristles are carefully removed, it will be seen that only 5 bristles are represented, each incised to the base into 3–5 lacineae. Occasionally entire bristles will be found, such as are normal in *P. ciliaris* (cf. Pl. 22, fig. 3b). In such cases, there is a corresponding reduction in number of lacineae to 8 or 9. Especially in ray flowers, scales are found which are identical with those of *P. angustifolia* Torr. The slender plants of my collections are the same as *Eggers* 15,785 from El Recreo, Ecuador, and *Weberbauer* 5889 from Serrán, Piura, Peru; the glomerate plants resemble *Stork* 11,368 from Huancabamba, Piura, Peru.

317. P. LINIFOLIA L. Syst. ed. 10, 1221. 1759; S. Moore in Fawcett & Rendle, Fl. Jam. 7, pt. 5: 266. 1936. An erect species with narrow elongate leaves and deep yellow rays, north slope of Puntilla, Salinas (11,128); river gravels, Quebrada Ancha, near La Brea, Peru (H&S 11,556). This is a widespread plant known from southwestern United


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States and the West Indies to western South America, and the only continental species known from the Galapagos Islands. In both of these collections there are 2–5 setae on the disc achenes.

318. PIQUERIA PERUVIANA (Gmel.) Robinson, Proc. Am. Acad. 42 [Contrib. Gray Herb. n. s. no. 32]: 13. 1906. *Flaveria peruviana* Gmelin, Syst. 2: 1269. 1791. A shrub 3–4 ft. high, with dark green deeply-dentate leaves, glabrous except for slight puberulence on the veins below. Rocky banks, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,537). The panicles (long past flowering) have ascending branches; the achenes are 1.4 mm. long. The species was originally described from Dombey's collection near Lima. Dr. Robinson has cited some collections from Alausi, Ecuador, the only example that I know of a native plant common to the Peruvian coast and the Ecuadorean Andes. This species is close to *P. densiflora* Benth. Bot. Sulphur 110. 1845 (Robinson, l.c.), which is known only from Puná Island, Ecuador, and which was described by Bentham as having membranous leaves, acuminate and "grosse dentatis," but the inflorescence stiff and glomerate at the ends of the divergent branches. *P. densiflora* is evidently represented by *Schimpff* 300 from rocky places in northern Guayaquil. The achenes are of the same structure and appearance as in no. 11,537 but are larger (1.7 mm. long) and the pappus disc is a little firmer and not at all erose.

319. PLUCHEA ODORATA (L.) Cass. Dict. Sci. Nat. 42: 3. 1826; S. Moore in Fawcett & Rendle, Fl. Jam. 7, pt. 5: 201, fig. 61. 1936. *Conyza odorata* L. Syst. ed. 10. 1213. 1759. Shrubs 4–5 ft. high in low ground between Ancon and Santa Elena (11,176) (det. Blake). Widespread in the tropics from Florida southward.

320. POROPHYLLUM MACROCEPHALUM DC. Prod. 5: 648. 1836; Millspaugh, Field Mus. Bot. 3: 141 (with fig.). 1904. Rydberg, N. Am. Fl. 34: 183. 1916. A strong-smelling weed on rocks and open headlands, Puntilla, Salinas (11,229); Punta Centinela (11,261); Quebrada Ancha, near La Brea, Peru (H&S 11,585). These plants have involucres 18–21 mm. long, and achenes 10–11 mm. long. The peduncles are less thickened than usual in this species, which, according to Rydberg, extends from Arizona to Panama, and is known from Ecuador. *Porophyllum ellipticum* (L.) Cass., which differs in having achenes 7–8 mm. long, and with involucres 18 mm. long, is reported from several islands of the Galapagos.

321. SIMSIA DOMBEYANA DC. Prod. 5: 578. 1836; Blake, Contrib. Gray Herb. no. 52: 44. 1917. A rough branching herb 0.5 m. high or less with inconspicuous pale yellow rays and rigid, almost spinescent chaff, in river gravels near La Brea, Peru (H&S 11,563). Coincides in all respects with the original description of Dombey's collection from Peru. The species is also known from Carthagena, Colombia, and from Argentina.

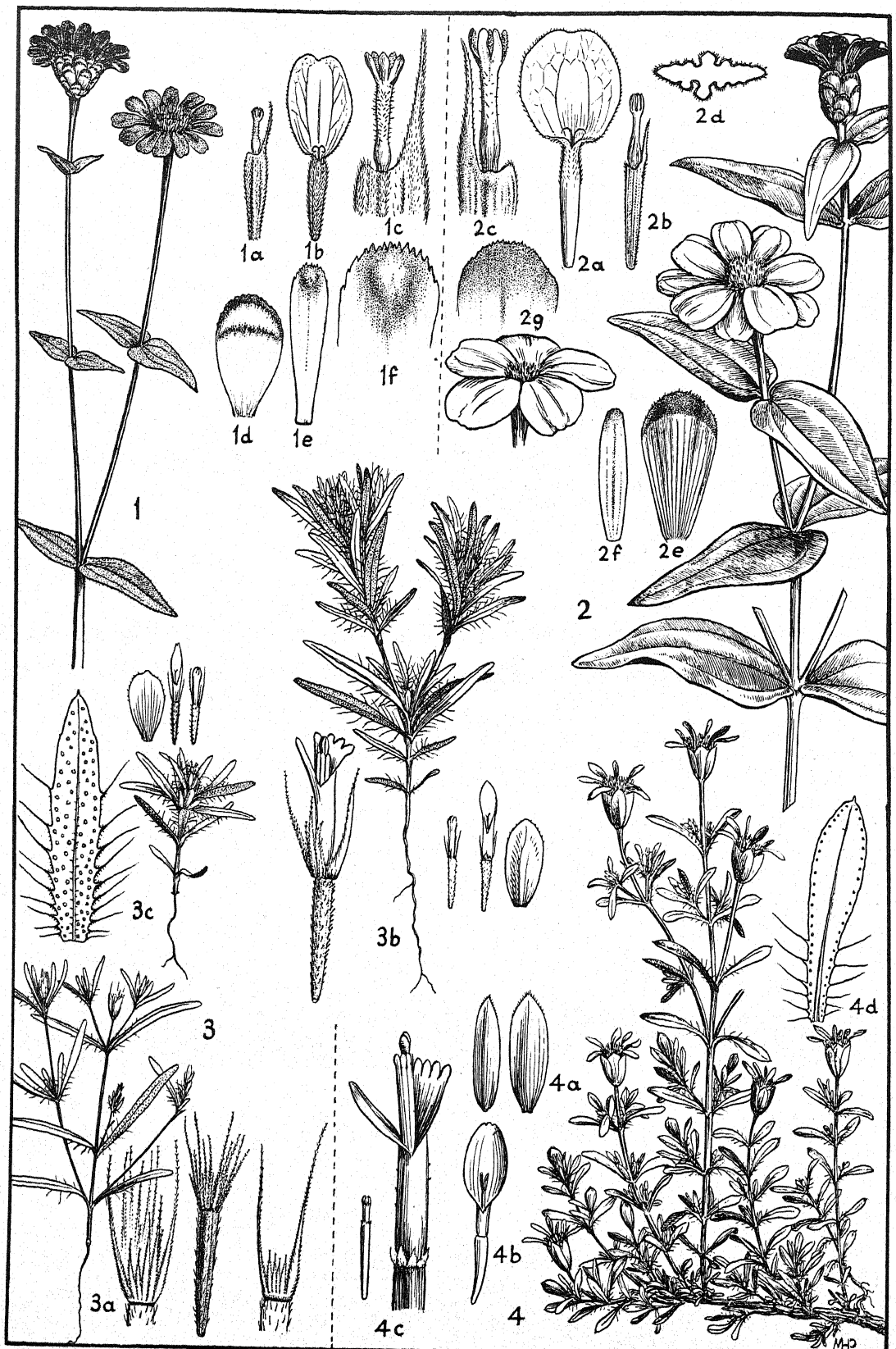
322. SPILANTHES ALBA L'Herit. Stirp. Nov. 1: 7, t. 4. 1785; Aiton, Hort. Kew. 3: 152. 1789. *Bidens ocymifolia* Lam. Encyc. 1: 416. 1785; Ill. Gen. 3: 244, t. 668, f. 3. 1797. *Spilanthes Salivaria* Murr. Comm. Goett. 3, t. 1. 1785. *S. ocymifolia* A.H. Moore, Proc. Am. Acad. 42 [Contrib. Gray Herb. n.s. no. 33]: 531. 1907.

Essentially glabrous herbaceous plants with shining deep-green leaves and rounded to somewhat elongate white heads in which the black anthers are conspicuous, rocky banks, Quebrada Mogollon, Amotape Hills, Peru (H&S 11,539); summit of Cerro Prieto, 2500 ft., Piura, Peru (H&S 11,617). It was also collected at Talara, *Haught* 108. In shaded thickets the white heads are mingled with blue flowers of *Browallia elata* and dull yellow *Zinnia peruviana;* all together make a rather colorful display in a region characterized by dullness of the vegetation.

Dombey got seeds of *Spilanthes alba* in Peru at an early date. By 1785 the species had been described from at least three botanic gardens, and in Hortus Kewensis (l.c.) it was said to have been introduced into England in 1783. Since all names appeared in the same year, and *S. alba* had current usage up to the time of Moore's paper, I think that name should be maintained. The Peruvian plant, even as to indication of the black anthers, is well shown in the Lamarck plate. How far northward typical *Spilanthes alba* extends is conjectural. The Mexican plant passing under this name appears to be wholly different. In the Peruvian plant the chaff is 4 mm. long, with a broad whitened apex, and is flat except for the lowest third of its length; the black flattened achene is 1.6–1.8 mm. long and 0.6–0.8 mm. wide and with two equal straight upwardly barbed pappus awns 0.6–0.8 mm. long (much longer than the marginal cilias), accompanied by several minute awns, and with a ciliate but wingless margin; the black anthers are 1.0 mm. long. In the Mexican plant (cf. *Pringle* 4341) the chaff is 5 mm. long, stramineous, and keeled to the apex; the black flattened achene is 2.0–2.2 mm. long and 1.0–1.2 mm. wide, usually with a single twisted awn about 1.5 mm. long, and a prominent pale brown winged margin in addition to the cilia, and with a prominent group of setae just below the apex; the pale brown anthers are about 0.5 mm. long. It is probable that more than a single species is involved in Mexico.

323. SYNEDRELLA NODIFLORA (L.) Gaertn. Fruct. 2: 456, t. 171, fig. 7. 1791; S. Moore in Fawcett & Rendle, Fl. Jamaica 7, pt. 5: 247, fig. 84. 1936. A plantation weed with inconspicuous yellow-rayed

Plate 21. *Wedelia grandiflora,* showing variation within the species.—Fig. 1. A narrow-leaved extreme (11,347, Ecuador) with 4-angled achene (1a).—Fig. 2. Three achenes (2a, 2b, 2c) (11,628, Ecuador), showing variability of pappus, and wings at achene-angles.—Fig. 3. A broad-leaved extreme (11,059, Ecuador) with characteristic pubescence of the species in detail (3a), and a ray-flower (3b).—Fig. 4. Intermediate form (*H&S* 11,628, Peru) with a ray-flower (4a) × 4; disc flower with stamen and style much enlarged (4b); and an involucral bract and a disc scale (chaff) enlarged (4c).


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heads, Manglaralto (11,479). A widespread species from Florida southward into the tropics; introduced in the Old World tropics.

324. VERBESINA EGGERSII Hieron. in Engler, Bot. Jahrb. 28: 612. 1901; S. F. Blake, Contrib. U.S. Nat. Herb. 26: 255. 1930. Exposed sea cliffs, Punta Ayangue (11,359); Punta Centinela (11,265); river alluvium, Pariñas Valley, Piura, Peru (H&S 11,517). Previously known only from El Recreo, Manabi Prov. (*Eggers* 14,941 & 15,687), and from Chanduy (*Spruce* 6472), (*Silphium platypterum* Spruce ex Benth. & Hook. Gen. Pl. 2: 350. 1873. nomen nudum). A slightly woody plant 3–5 ft. high, with white rays, and coarsely-serrate lanceolate leaves (to 7 cm. broad) with a non-serrate cuneate base. My Ecuadorean specimens are somewhat coarser than the type, and the heads often 1 cm. high. In the Peruvian collection they are 5–8 mm. high, but the general appearance of the plants and the structure of the achenes is the same. The achene surface varies from smooth to coarsely scabrous. Hieronymus considered this species as related to *V. Humboldtii* Spreng. (*V. helianthoides* HBK.) (cf. Robinson, Proc. Am. Acad. 34 [Contrib. Gray Herb. no. 16]: 564. 1899), a plant which is supposed to have come from Ecuador.

325. VERNONIA BACCHAROIDES HBK. Nov. Gen. & Sp. 4: 38. 1820. Bushes, or more frequently trees to 30 ft. high, Manglaralto (11,460, det. S.F. Blake), and extending eastward along the Chongon Hills to Guayaquil (*Mille* 220). The white pappus of the flowers is conspicuous at a distance. The species was originally described from the Andes of Colombia.

326. WEDELIA GRANDIFLORA Benth. Bot. Sulphur 115. 1845. *W. Eggersii* Hieron. Bot. Jahrb. 28: 604. 1901. A very abundant shrub forming thickets in low ground east of Santa Elena, and reaching the coast at Punta Ayangue (11,347); summit of Chanduy Hills, 1800 ft. (11,041 & 11,059, a broad-leaved form); in ravines, Pariñas Valley (H&S 11,522); and in stream beds at base of Cerro Prieto, Piura, Peru (H&S 11,628) (Pl. 21). I have seen the following additional collections: ECUADOR: Oil Camp between Guayaquil and Salinas (*Hitchcock* 20, 029); western Guayaquil (*Schimpff* 404); El Recreo, Manabi (*Eggers* 14, 972, type collection of *W. Eggersii*). PERU: Pariñas Valley (*Haught* 91); mts. east of Hacienda Chicama, Tumbez (*Weberbauer* 7658).

Wedelia grandiflora is close to *W. helianthoides* which came from dry hills between Alausi and Tixan, in the central Cordillera of Ecuador, but has larger flowers. *W. helianthoides* extends to Paraguay (cf. Hassler, Rep. Spec. Nov. 14. 178. 1915). The original collection of *W. grandiflora* came from Guayaquil. The branchlets were described as hirsute toward the apex, with long soft white hairs; the ligules 15–17 mm. long, and the leaves 2 inches broad. There is great variation in plants growing in exposed situations, such as no. 11,347 from Punta Ayangue, with scabrous branches and leaves not more than 3 cm. wide, to the thin-leaved soft phase with leaves as much as 11 cm. wide (no. 11,059) from moist thickets in the Chanduy Hills (cf. Pl. 21). The last-named collection probably represents var. *macrophylla* Bentham (l.c.).

A mature collection from Piura (H&S 11,628) is lightly scabrid and has rays up to 20 mm. long; the outer achenes are sharply quadrate with the two outer angles somewhat prolonged, and a fringed cup-like structure, elevated by a neck, represents the actual pappus. A few bristles are frequently somewhat longer than the others. The inner achenes are flattened, and do not have the prolonged outer angles characteristic of the marginal achenes. This shrub is not known from the Galapagos Islands.

327. W. TRILOBATA (L.) Hitch. Rept. Missouri Bot. Gard. 4: 99. 1893; S. Moore in Fawcett & Rendle, Fl. Jamaica 7, pt. 5: 227, fig. 75. 1936. Stream bank, Manglaralto (11,584). A variable species of tropical America, known from Florida southward to Colombia.

328. ZINNA PERUVIANA L. Syst. Nat. 10. 1221. 1759. *Chrysogonum peruvianum* L. Sp. Pl. 920. 1753. *Bidens, calyce oblongo* . . . . Mill. Dict. 43, t. 64. 1756. *Rudbeckia, foliis oppositis* . . . . Zinn, Goett. t. 1. 1757. *Zinnia pauciflora* L. Sp. Pl. ed. 2. 1269. 1763 (renaming of *Z. peruviana*); Lam. Encyc. 8: 861. 1808. *Z. lutea* Gaertn. Fruct. 2: 459, t. 172, f. 3. 1791 (renaming of *Z. pauciflora* L.); Lam. Encyc. t. 685, f. 2. 1797.

Scattered in rocky woods or dry fields, ridge north of Santa Elena (11,101); fields north of Punta Carnera (11,295); Punta Centinela (11,391); vic. La Brea, Piura, Peru (H&S 11,579). In Ecuador this plant was just coming into bloom on February 22 and I collected it as late as April 6, but none of these collections were mature, whereas the material obtained in Peru on March 29 had mature achenes. In general the vegetation of the Ecuadorean coast was ten days to two weeks behind that of Peru, due probably to the relatively

Plate 22.—Fig. 1. *Zinnia multiflora* (*Macbride* 3160, Peru), disc flower (1a) × 1.5 and ray flower (1b); enlarged apex of disc flower (1c); involucral bract (1d); disc chaff (1e), with enlarged apex (1f).—Fig. 2. *Zinnia peruviana* (11,295, Ecuador) structural details from *Haught & Svenson* 11,579: ray flower (2a) × 1.5; disc flower (2b), with enlargement (2c); cross-section of achene (2d); involucral bract (2e); chaff from disc (2f), with apex enlarged (2g). Note less-pubescent achenes and flowers of *Z. peruviana*, double-banded apex of involucral bracts, and shallow indentation of the chaff apex.—Fig. 3. *Pectis ciliaris*, a series of collections to show variation in habit, pappus, and pubescence of achenes. Expanded form (11,124, Ecuador) (3a); achenes with various types of pappus. Congested form (11,267, Ecuador) (3b). To right × 1.5 disc and ray flower, and involucral bract; to left enlarged disc flower. Dwarfed form (11,096, Ecuador) (3c), with enlarged leaf.—Fig. 4. *Pectis arenaria* (11,200, Ecuador), with involucral bracts (4a), a ray flower (4b), a disc flower (4c) × 1.5, also with enlargement; an enlarged leaf (4d).

greater amount of cloudy weather in Ecuador. These collections are identical with Zinn's and Miller's figures, representing plants grown from seed obtained by Joseph Jussieu probably between 1735 and 1740 in Peru (or possibly near Guayaquil, where Jussieu was in 1735). Miller (l.c.) got seeds in 1753, the plant having been grown for some time previously at Paris. Haught first collected this plant which appears in Peru after the occasional rains, sometimes (as in March, 1941) covering the ground almost by the acre, from the Mancora Valley, 50 mi. south of Tumbez (no. 153, April 16, 1927). The rays are always broadly obovate and a light to a deep, rather dull yellow. The sparse appressed pubescence becomes more abundant and somewhat silky just under the inflorescence. Some of the plants exceed two feet in height, although that is exceptional, and Miller mentioned them as becoming four feet tall under cultivation. This plant differs from *Z. multiflora* in having constantly yellow rays, with the ray-achenes pubescent only near the apex. The darkened apex of the involucral bracts is much narrower, the chaff is practically toothless, and the corollas are larger. In *Z. multiflora* the chaff-apex is prominently toothed; in *Z. elegans* it becomes deeply crested. In the original publication Linnaeus included a reference to Feuillé, but this reference (evidently descriptive of a species of *Bidens*) was removed in the second edition of Species Plantarum. Considering the ephemeral character of the vegetation of northwestern Peru, it is not surprising that true *Zinnia peruviana* remained unrecognized for nearly two centuries (Pl. 22, fig. 1 & 2).

SUMMARY

The vegetation of the Galapagos Islands is closely related to that of the South American mainland, but analysis does not suggest continental land connections in the past. Kroeber (1916), reviewing the opinions of Baur, together with additional data from collections by Stewart (1911), states, "In general the number of species common to any two islands depends not so much on their location relative to each other, nor on their altitude, rainfall, or position in certain winds or currents, but on the mere wealth and variety of their plant lives." Wealth of plant life on the individual islands of the Galapagos depends largely on altitude (which determines the amount of precipitation) and on area (which allows development of isolated species in favorable pockets).

Stewart's estimate of 40 per cent endemism in the plants of the Galapagos Islands seems too large. It was based on the assemblage not only of species, but of the varieties and forms, of which there is no end if subdivision is carried to an extreme. Thirty-five species, treated as endemic by Stewart and later writers, are considered as not being confined to the Galapagos Islands. Probably the Galapagos endemism is not relatively greater than that of the rain-green forests, and associated arid scrublands about the Gulf of Guayaquil in Ecuador and Peru, with which the vegetation of the Galapagos Islands has much in common. The reader should compare the various lists of species cited under the heading "Geographical Distribution of the Galapagos-Gulf of Guayaquil Flora."

Vegetation in the Galapagos Islands is characterized by "dwarfing," which involves reduction of leaf surface from locality to locality, probably in response to ecological conditions. This situation does not appear markedly in the analogous zone of the mainland, but is characteristic of some West Indian islands. It is strikingly seen in *Croton, Sida, Alternanthera, Lantana, Acacia,* and some other genera. The Galapagos Islands have the same general temperature, rainfall, and fog conditions encountered in the arid region of the Gulf of Guayaquil (illustrated by comparative charts in the text), but they differ in one climatic respect. They have only in small part the intensely drying southwest winds which blow steadily from the Pacific Ocean across the continental lowlands. In comparison with the desert flora from Santa Elena, on the Ecuadorean coast, and from the coast of Peru, the Galapagos flora is luxuriant. In the Galapagos Islands, plants exist on seacoasts and recent lava flows, that could not live at all on the dry Peruvian coast. Once established, they survive in the Islands, though often in a state where transpiration must be reduced to a minimum. Or they may spread out into the wealth of ecological environments open to them. A xerophytic forest, similar to that of the Guayaquil region, though greatly reduced in number of species, occupies the windward slopes of the higher islands in the Galapagos Archipelago. This zone is followed, higher up, by a scrub forest rich in ferns, and on one or two of the islands, by a dry grassy zone above the general cloud level.

The coastal area of the South American continent which agrees with the climate of the Galapagos Islands—except perhaps for local spots in the Ica to Mollendo region of southern Peru in the zone of heavy mists or garuas—is the coast of Ecuador from Cape Pasado, just below the equator, to Santa Elena, encompassing only two degrees of latitude. The Galapagos Islands cover approximately three degrees. The Islands are geographically intermediate between the dry coast of Ecuador and Peru and the dry northern coasts of Colombia and the xerophytic islands of the West Indies. Thirty species are recognized as occurring only in the Galapagos Islands-Gulf of Guayaquil region, while only two (*Psidium galapageium* and *Cyperus pycnostachyus*) are confined to Mexico and the Galapagos Islands, and a single species (*Euphorbia viminea*) to the Galapagos and the West Indies (Bahamas). None are restricted to the Galapagos Islands and the north coast of South America. Nevertheless, it is evident in *Alternanthera,* that, although the bulk of the Galapagos species are from the adjacent coast of South America, one of the species (*A.*

ficoidea) is from the Caribbean region. The Bahama species of *Borreria* though superficially similar to those of the Galapagos Islands, are entirely distinct. With the rain-forest coast of South America from the equator to Panama, the vegetation of the Galapagos Islands has almost nothing in common, except for widely-distributed plantation weeds. The flora of the Galapagos Islands would seem to be of comparatively recent introduction.

The annotated catalogue comprises 328 species of which six are new: *Opuntia melanosperma, Brachistus Haughtii, Solanum amotapense, Solanum talarense, Heliotropium piurense* and *Ruellia pacifica*. Several genera—especially *Alternanthera, Prosopis, Tephrosia, Sida,* and *Ipomoea*—include a comparative study of West Indian species.

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SOME NEW OR OTHERWISE NOTEWORTHY DICOTYLEDONOUS PLANTS [1]

Earl Edward Sherff

DODONAEA **nematoidea** sp. nov.—Frutex ±2 m. altus; ramulis griseis, subteretibus, longitudinaliter strigosis porriginosisque, inferne efoliatis. Folia glabrata, utrinque acuminata, griseo-viridia, ad ramulorum apices conferta, basi in petiolum gracilem ±1 cm. longum attenuata, lamina (7–11 cm. longa et 1–2 cm. lata) anguste elliptico-oblonga vel oblanceolata interdum obscure repandula. Florae sine dubio dioicae (tantum pistillatae visae), numerosissimae, in inflorescentia glabrata sub 8 cm. longa lataque dispositae, gracillimae. Pedicelli nematoidei, ±1 cm. longi. Sepala 3–5, ovata, plus minusve hispidula, 1–3-nervata, circ. 2.5 mm. longa, patentia reflexave. Ovarium obovatum, glabratum sed plus minusve gummiferum. Stylum gracillimum itaque nematoideum, plerumque 6–12 mm. longum, duplex. Fructus non visi.

Shrub ±2 meters tall; branchlets gray, subterete, lengthwise strigose and scurfy, leafless below. Leaves glabrate, acuminate at both ends, grayish-green, crowded at tips of branchlets, narrowed at base into a slender petiole ±1 cm. long; blade (7–11 cm. long and 1–2 cm. wide) narrowly elliptic-oblong or oblanceolate, at times obscurely subrepand. Flowers without doubt dioecious (only the pistillate seen), very numerous, very slender, disposed in a glabrate inflorescence, this under 8 cm. long and wide. Pedicels thread-like, ±1 cm. long. Sepals 3–5, ovate, more or less hispidulous, 1–3-nerved, about 2.5 mm. long, spreading or reflexed. Ovary obovate, glabrate but more or less gummiferous. Style very slender and thus thread-like, commonly 6–12 mm. long, double. Fruits not seen.

Specimens examined: *C. T. White* 7,679, large shrub 2 meters tall, flowers greenish, common in rocky gorge base of Biggenden Bluff, Biggenden, South Burnett District, Queensland, May 17, 1931 (type, Arn.: isotype, N.Y.).[2]

The specimens had been determined as *Dodonaea viscosa.* The leaves do resemble those on some specimens of *D. viscosa* var. *linearis* f. *angustifolia.* The hair-like or thread-like pedicels and styles are, however, quite different from those of the approximately two thousand specimens of *D. viscosa* and its various forms examined by me.

STELLARIA MEDIA (L.) Vill. Hist. Pl. Dauph. 3: 615. 1789;[3] *Alsine media* L. Sp. Pl. ed. 1: 272. 1753.—The genus *Stellaria* L. apparently was unknown from the Hawaiian Islands in Hillebrand's day, for in his Flora he omitted all reference to it. The genus has been found there since then, however (*cf.* Univ. Hawaii Occas. Papers no. 36: 30. 1938). Recently I have seen two Hawaiian specimens of *S. media: Otto Degener* 5,958, west side of Pohakunui Gulch, Isl. Molokai, May 6, 1928 (Chi.); *Albert S. Hitchcock* 14,409, alt. 6,000 feet, moist, wooded hill, Puu Huluhulu, Humuula Sheep Station, Isl. Hawaii, Aug. 27, 1916 (U.S., under name *Silene gallica* L.).

SILENE STRUTHIOLOIDES **typica** var. primum nominata; *Silene Struthioloides* var. *α* A. Gray, Bot. United States Explor. Exped. 1: 110 & *tab.* 10, A. 1854–1857.—Asa Gray founded his species *S. Struthioloides* upon specimens collected by the U. S. Exploring Expedition on the Island of Hawaii. He described two varieties, designated by letter only. For his var. *α*, here named var. *typica,* he gave, "foliis rigidioribus cum ramis et inflorescentia glaberrimis." His var. *β* he described with the words, "foliis tenuioribus saepius longiorbius, juventis praesertim ad margines ramulisque pl. m. viscoso-pubescentibus." Hillebrand (Fl. Haw. Isls. 28. 1888) unfortunately overlooked or ignored Gray's varieties. Skottsberg, in a recent paper (Haw. Vascular Pl. IV: 368, Meddel. Göteborgs Bot. Trädgård 15: 368. 1944), referred certain Maui plants to Gray's var. *α* and some from Kilauea on Hawaii to Gray's var. *β.* He made no conventional name, however, for either variety.

Herbarium specimens of *Silene Struthioloides* (so far as known to me), other than a single suite, Degener 2,313 from Kaupo Gap on East Maui, all are sharply separable into the two varieties noted by Gray and without question merit regular varietal names, in accordance with customary present-day practice. Var. *typica* is seen to occur in several localities on Hawaii and East Maui. Var. *β,* named var. *gracilis* below. is limited to Hawaii. *Degener* 2,313, described below as var. *kaupoana,* is limited so far as is known to a small area at the

[1] Received for publication January 23, 1946.

[2] The following abbreviations are used for the depositories cited in this paper: Arn., Arnold Arboretum, Jamaica Plain; Berl., Berlin Botanical Garden; Calif., University of California, Berkeley; Chi., Chicago Natural History Museum (formerly Field Museum of Natural History), Chicago; Del., Delessert Herbarium, Geneva; Goth., Arboretum (Botanical Garden) of Gothenburg; Gray, Gray Herbarium of Harvard University, Cambridge; Kew, Royal Botanical Gardens, Kew; Len., Botanical Garden of Leningrad (Hortus Petropolitanus); Mex., National University of Mexico, City of Mexico; Mo., Missouri Botanical Garden, St. Lou's; Mun., Botanical Garden of University of Munich; Mus. V., Natural History Museum of Vienna; N.Y.; New York Botanical Garden; Par., Museum of Natural History, Paris; Penn., University of Pennsylvania; Phila., Academy of Natural Sciences, Philadelphia; U.S., United States National Museum, Washington, D.C.; U.V., University of Vienna.

[3] Various authors have cited Cyrilli (Cyrillo), De Ess. Pl. Char. Comm. 36. 1784. As pointed out by certain workers, however, Cyrilli "did not absolutely conjoin the generic and specific names" (*vide* Britten, Jour. Bot. 37: 38. 1899). Mr. F. N. Williams accordingly (*cf.* Britten, *loc. cit.*) accredits Villars with the combination *Stellaria media.* In this he was very properly followed by Graebner (in Aschers. & Graebn. Synops. Mitteleur. Fl. 5[1]: 527. 1919), in the latter's comprehensive treatment of the Caryophyllaceae of central Europe.


#3808
Herb. Kew.

northeast corner of Haleakala Crater on East Maui.

Specimens examined (for var. *typica*) East Maui: *Otto Degener* 17,154, lava region often cloud-swept, near Koolau Gap, Haleakala, June 29, 1927 (Chi.; N.Y.); *Degener* 17,158, dry place, Pipe-line Trail, Olinda, June 27, 1927 (N.Y.); *Degener & Henry Wiebke* 2,312 in volcanic, gravelly ash near cinder-cones in Haleakala Crater, Aug. 15, 1927 (Calif.; Chi.; Kew; N.Y.; U.S.); *Charles Noyes Forbes* 280-M, Crater of Haleakala, August, 1910 (Calif.; Kew; Mo.; N.Y.; U.S., 2 sheets); *William Hillebrand* 422 *pro parte,* alt. 7,000–9,000 feet, Haleakala, July, 1858 (Gray; Kew, 2 sheets); *Joseph F. Rock* 8,587, Puuniauniau (Puu Nianiau), Haleakala, October, 1910 (Gray).

Island of Hawaii: *David Douglas* 2 (Gray; Kew; N.Y.; U.S.);[4] *C. N. Forbes* 889-*H,* at source of Wailuku River, June 18, 1915 (Kew; Mo.; N.Y.; U.S.); *Albert S. Hitchcock* 14,292, in loose lava, alt. 8,000–11,000 feet, on slope, north side of Mauna Kea, Aug. 22, 1916 (U.S.); *Joseph F. Rock* 8,300, alt. 10,500–11,000 feet, Papalekoki, Mauna Kea, June, 1910 (Calif.; Gray; Kew; N.Y.; U.S.); *U.S. (South Pacific) Explor. Exped. under Capt. Wilkes* ["on Mouna Loa, and Mouna Kea . . . . in the region of *Edwardsia* (8,000 feet and higher); near the crater of Lua Pele; also in the district of Waimea, in the same island" *fide Grayi loc. cit.*], 1838–1842 (Gray, *cum.* var. *β,* A. Gray *commixt. ut etiam in* N.Y. *et* U.S.).

Silene Struthioloides **gracilis** var. primum nominata; *Silene Struthioloides* var *β* A. Gray *loc. cit.*—Specimens examined (all from Island of Hawaii): *Otto Degener,* Kau Desert, August, 1923 (Chi.); *Degener* 17,153, dry, bare lava region, Uwekahuna, Kilauea, June 18, 1929 (Mo.; N.Y.); *Degener* 17,155, lava wastes, Kau Desert, July 15, 1926 (Mo.; N.Y.); *Charles N. Forbes* 204*H,* summit of Hualalai, June 19–21, 1911 (Kew; N.Y.); *Forbes* 248-*H,* Kanehaha, Kau, June 23, 1911 (Calif.; Chi.; Kew; Mo., 2 sheets; N.Y.; U.S.); *William Hillebrand* 422 *pro parte* (Gray; Kew); *Horace Mann, Jr., & William T. Brigham* 312, alt. 8,000 feet, Hualalai (Chi.; Gray; U.S.); *Mann & Brigham* (similarly) 312, Kilauea (Chi.; N.Y.); *Miss Marie C. Neal,* Kilauea, near Keanakokoi Crater, Aug. 2, 1927 (Calif.); *Joseph F. Rock,* Puulehua (Gray, 2 sheets); *Rock,* Kilauea Volcano, in 1911 (Gray, 2 sheets); *W. A. & C. B. Setchell,* Kilauea, July 2, 1924 (Calif.); *U.S. (South Pacific) Explor. Exped. under Capt. Wilkes,* 1838–1842 (type collection of var. *β* A. Gr., Gray; N.Y., 2 sheets; U.S.).

The Hooker herbarium at Kew has one sheet bearing six specimens collected by Hillebrand. They all bear Hillebrand's no. 422. Two came from Haleakala and are var. *typica.* The other four came from Hawaii (doubtless Hualalai, the third of his locality citations for Hawaii, Gray having originally made the first two). These four are var. *gracilis.* In Hillebrand's text he noted that "in the Maui plants the branch passes gradually into the inflorescence, while in those from Hawaii the two are rather distinct." This difference is somewhat discernible among these six specimens but does not hold well in general (*cf.* Skottsberg, *loc. cit.,* who says that it is not observed in his own specimens).

The description for this variety is quoted above under var. *typica.* To it we may add that the stems and branches are more or less densely viscid-pubecent with retrorse hairs.

Silene Struthioloides **kaupoana** Degener & Sherff var. nov.—Omnino glaberrima vel foliorum basibus raro sparsim hispida. Folia patentia vel recurvata, tenerrime filiformia, ±1.5 cm. longa, acerrime sed aegre apiculata. Ovarii stipes longior (7–10 mm.).

Very glabrous throughout or rarely somewhat hispid at the bases of the leaves; these spreading or recurved, very slenderly filiform, ±1.5 cm. long, very sharply but weakly pointed at tip. Gynophore longer (7–10 mm.).

Specimens examined: *Otto Degener* 2,313, cliffs, (drooping from rock crevices on north side of) Kaupo Gap, Haleakala, East Maui, Aug. 20, 1927 (type, N.Y.: isotypes, Chi.; Gray; Kew; Mo.; Phila.).

All other specimens of *S. Struthioloides* examined thus far from the Island of Maui are referable clearly to the var. *typica,* which differs sharply in its more erectly and numerously branched, *Juniperoid* habit, its broader, less recurved, more bluntly and more callous-tipped leaves, and shorter stipe or gynophore, measuring only 4–6 mm. long.

Silene lanceolata **typica** var. nov.; *Silene lanceolata* A. Gray, Bot. United States Explor. Exped. 1: 111 and *tab.* 10, B. 1854–1857 (*sensu stricto, plantis mauiensibus exclusis*).—For his *Silene lanceolata,* Asa Gray cited specimens (1) from "the mountains of Kauai" and (2) from "Maui (specimen without flowers or fruit)." His description of flowers and fruits was drawn necessarily, of course, from the Kauai material. Even for the leaves, which he described as " . . . . sessile, *narrowed towards the base,* where *they are ciliate,* otherwise entirely glabrous, rather coriaceous in texture, flat, spreading, 1½ to 2½ inches long, about 3 lines wide . . . ," he relied upon the Kauai plants, to a great extent ignoring material from

[4] The Douglas labels do not state upon which island he collected his no. 2, but there is no doubt that it was Hawaii, where he immediately started collecting when he arrived in the Hawaiian Islands and remained until his abrupt death (*vide* H. Mann, Enum. Haw. Pl., Proc. Amer. Acad. 7: 144. 1867).

Fig. 1. *Silene lanceolata* var. *Hillebrandii,* from type sheet in Herb. Kew (photograph by author, his negative no. 3,803).

Maui, which latter he had no positive means of identifying definitely, even as to genus.[5] One sterile Maui spray examined by me (U.S., herb. no. 8,973, mounted on sheet with the very Kauai spray from which Gray's type illustration of *S. lanceolata* appears to have been drawn) has a *Schiedea*-like aspect and is clearly *Schiedea implexa* (Hillebr.) Sherff, a species native to both East and West Maui. Its leaves are up to 5 inches or over in length and to a full ½ inch in width, definitely membranaceous, strongly attenuate at both ends, and tend to show a slender petiole about ¼ inch long.

Other material has been suspected by certain workers as representing *S. lanceolata*, but I have seen no specimens other than those of the type collection from Kauai which could be reconciled with Gray's description. These, for greater precision, may be set off as the var. *typica*.

Hillebrand (Fl. Haw. Isls. 28. 1888) gave one additional variety, with the meager words: "*var. angustifolia.* Leaves linear, 1″ [*i.e.*, 1 line] broad." He cited no habitat nor have I seen representative material. For his treatment of *S. lanceolata* proper, however, he added to the "Kauai and Maui" of Gray the words: "*Central Plateau* of Hawaii! (Hbd.); Lanai! (Lydg.); at elevations of 300–500 ft." Material from Lanai is not at hand, but fortunately there is before me, from Hooker's herbarium at the Royal Gardens of Kew, a very full sheet of flowering and fruiting material from the Central Plateau of Hawaii. It is of Hillebrand's own collecting and bears his number 423. It had been "received July, 1865." It differs at once from var. *typica* in its mostly broader leaves, its pubescent calyces, its mostly eciliate bracts,[6] and, what is doubtless much the most trustworthy character, its elongate pedicels. It may be distinguished here as:

Silene lanceolata **Hillebrandii** var. nov.—Folia nunc linearia et 3–6 mm. lata nunc (saltem caulina) oblonge lanceolata vel saepius oblonge oblanceolata et 8–14 mm. lata. Inflorescentia usque ad 50–80-flora; bracteis plus minusve eciliatis; calycibus pilosis vel puberulentis; pedicellis longioribus (saepe 1–1.5 cm.), puberulentis (Fig. 1).

Leaves now linear and 3–6 mm. wide, now (at least those of the stem) oblong-lanceolate or more often oblong-oblanceolate and 8–14 mm. wide. Inflorescence up to 50–80-flowered; bracts more or less eciliate; calyces pilose or puberulent; pedicels longer (often 1–1.5 cm.), puberulent.

Specimens examined: *William Hillebrand* 423, Central Plateau, Isl. Hawaii (type, Kew).

Silene lanceolata **Forbesii** var. nov.—A var. *typica* differt: habitu humiliore simplicioreque, plantis 2–4 dm. altis; foliis nunc angustissime linearibus (2–4 mm. latis) nunc anguste oblanceolatis (circ. 9 mm. latis); bracteis aegrius ciliatis; pedicellis gracilioribus longioribusque (saepe 8–13 mm.); calyce paulo longiore, urceolato-cylindrico (non demum subcampanulato); capsula angustiore.

Differing from var. *typica* in its lower height and less branched habit, 2–4 dm. tall; leaves now very narrowly linear (2–4 mm. wide) now narrowly oblanceolate (about 9 mm. wide); bracts more weakly ciliate; pedicels longer and more delicate (often 8–13 mm. long); calyx a little longer, urceolate-cylindric (not finally subcampanulate); capsule more slender.

Specimens examined: *Charles Noyes Forbes* 131-*Mo*, on slopes below Puu Kolekole, southeastern Molokai, July, 1912 (type, Mo.: isotypes, Calif.; Chi.; Gray; Kew; N.Y.; Phila.; U.S.).

The Forbes collection had been distributed from the Bishop Museum in Honolulu under the name *Silene Alexandri* Hillebr. That species had indeed been described from a near-by locality ("*Kamalo*, about 2000 ft. above the sea") but is seen to differ sharply in having a clavate-elongate calyx, a much longer stipe or gynophore, and claws of the petals protruding from the calyx. The affinity of the Forbes plants is clearly with *S. lanceolata*, from the var. *typica* of which they differ readily in the characters noted. In var. *Forbesii* the leaves are at times extremely narrow, attaining the narrowness (1 line) given by Hillebrand for his var. *angustifolia*.[7]

Silene **Degeneri** sp. nov.—Fruticosa, decumbens vel adscendens ramis erectis; caulibus ramisque 2–4 mm. crassis, dense molliterque viscoso-pubescentibus, usque ad 4 dm. altis, fere vel omnino usque ad inflorescentiam viscido-pubescentem foliosis. Folia nunc pauca principalia oblonge linearia vel lineari-lanceolata, basi lata sessilia, apice acerrime caudato-attenuata, faciebus adpresse hispidula vel demum glabrata, marginibus numerose tuberculato-denticulata (itaque non vere integra) etiam elongate graciliterque ciliata, circ. 2.5–3.5 cm. longa et 2.5–7 mm. lata; alia vel nunc omnia similia sed multo minora (±dimidio breviora angustioraque), numerosissima, omnia patentia vel demum demissa; axi'lis plerumque surculis abortivis instructis. Florae (fide lectorum fragrantes) racemo-paniculatae, graciliter pedicellatae pedicel-

[5] Curiously, however, Gray described the leaves as lanceolate, even naming the species "*lanceolata*." While they are indeed more or less lanceolate in the sterile Maui plants (which are to be excluded from the species and even from the genus), they are more properly termed linear in the Kauai plants.

[6] In the type collection of var. *typica* the bracts of the inflorescence are very noticeably shaggy-ciliate, with many of the cilia appearing under a lens jointed or multiloculate; the flowers, although described by Gray as "numerous, rather crowded," would number 15 to 30 in an inflorescence.

[7] As already pointed out, I have seen no authentic var. *angustifolia* material, nor does the Hillebrand herbarium, once extant in Berlin, seem ever to have contained any. Hillebrand's text makes no mention of Molokai (the home of var. *Forbesii*) for *S. lanceolata* specimens, however, and it appears therefore that he could never have had var. *Forbesii* in mind. Nor indeed is it credible that so keen an observer as he would have failed to note the quite differently shaped calyces and capsules of var. *Forbesii* if he had really had them before him.

lis 5–15 mm. longis, bracteis subulatis. Calyx primum clavatus, demum inferne graciliter obconicus superne dilatatus et suburceolatus vel fere subcampanulatus, 1.5–1.9 cm. longus, pubescens, infra manifeste supra obsolete 10-nervis, lobis triangulato-ovatis apice acutis. Stipes (gynophorum) gracilis, circ. 6–7 mm. longus. Petala bifida, laminis 4–7 mm. longis, facile (2 usque ad 5 mm.) exsertis. Capsula anguste ovoidea, sepala demum superans (Fig. 2).

Shrubby, decumbent or ascending with erect branches, these and the stems 2–4 mm. thick, densely and softly viscid-pubescent, up to 4 dm. tall, leafy almost or completely up to the viscid-pubescent inflorescence. Now a few principal leaves oblongly linear or linear-lanceolate, sessile at their broad base, very sharply caudate-attenuate at apex, appressedly hispidulous on their faces or finally glabrate, at margins numerously tuberculate-denticulate (therefore not truly entire) and also elongately and delicately ciliate, about 2.5–3.5 cm. long and 2.5–7 mm. wide; the others or now all similar but much smaller (±half as long and wide), exceedingly numerous; all spreading or finally drooping, their axils mostly bearing abortive shoots. Flowers (fragrant according to the collectors) racemo-paniculately disposed, slenderly pedicellate, pedicels 5–15 mm. long, bracts subulate. Calyx at first clavate, at last slenderly obconic below but dilated and suburceolate or almost subcampanulate above, 1.5–1.9 cm. long, pubescent, prominently 10-nerved below but obsoletely so above, the lobes triangulate-ovate, their apex acute. Stipe (gynophore) slender, about 6–7 mm. long. Petals bifid, their blade 4–7 mm. long and exserted as much as 2–5 mm. Capsule narrowly ovoid, finally surpassing the sepals.

Specimens examined: *Otto Degener & Henry Wiebke* 2,310, over dry, rocky embankment in Haleakala Crater near Koolau Gap, East Maui, Aug. 9, 1927 (type, N.Y.: isotypes, Calif.; Chi.; Kew; N.Y.; U.S.).

The type collection was distributed as *Silene lanceolata* A. Gray. That species differs sharply in having fewer leaves, with no semblance of minute teeth along their edges, a very short gynophore, a campanulate calyx, more completely included petal-claws, etc. As already pointed out, true *Silene lanceolata* is unknown from the Island of Maui, Gray's (and Hillebrand's) citation of Maui tracing back to certain sterile sprays of *Schiedea implexa* (Hillebr.) Sherff collected by the U. S. Exploring Expedition on Maui and erroneously associated by Gray with his *Silene lanceolata*.

It is a pleasure to name this remarkable species after Mr. Degener, who has done so much to expand our knowledge of Hawaiian plants.

SILENE GALLICA L. Sp. Pl. ed. 1: 417. 1753; Graebner (*pat. & fil. in* Aschers. & Graebn. Synops. Mitteleur. Fl. 5^{II}: 96. 1929.—For this species several botanists have preferred the name *Silene anglica* L., a name published by Linnaeus (*loc. cit.* 416) one page earlier than or ahead of his *S. gallica*. The two names are indeed universally regarded today as synonymous *specifically* (though not *varietally*), but apparently the first botanist to note formally their specific identity chose to retain *S. gallica* as the name to use.[8]

Graebner & Graebner (father and son) are among those to retain *S. gallica*. For it they present (*loc. cit.*) an extended conspectus of subordinate forms and races. These they divide into two main groups: A, those having capsules erect or suberect at maturity and B, those having capsules horizontal or turned backward at maturity.[9] Under A is found, as equivalent to one (*i.e., silvestris*) of the two component races, *S. gallica* proper (*i.e.,* f. *genuina* Rohrb.), while *S. anglica* is given as one (*i.e., anglica*) of the two component races of B.

All specimens examined from the Hawaiian Islands have erect or suberect capsules at maturity and may be considered *S. gallica* in its narrower sense.

Specimens examined: Island of Kauai.—*Otto Degener* 5,955, dry region, Waimea Canyon near Kokee Camp, June 30, 1926 (Gray; N.Y.).

Island of Oahu.—*Degener* 2,256, arid slope, Kolekole Pass, October, 1925 (U.S.); *Degener* 2,291, exposed rocky ridge, same place and date (Chi.); *Degener* 5,973, sand dunes, Waimanalo, Apr. 13, 1927 (N.Y.; U.S.); *A. A. Heller* 2,202, on lower slopes of Konahuanui, above Manoa, Apr. 22, 1895 (N.Y.; U.S.); *Heller* (similarly) 2,202, along the roadside, as well as clinging in crevices, high up on the cliffs, Nuuanu Pali, Apr. 23, 1895 (Calif.; Chi., 2 sheets; Gray); *Heller* (similarly) 2,202, Nuuanu Pali, May 24, 1895 (Kew); *D. L. Topping* 3,136, Puu Kaua, May, 1925 (Calif.; N.Y.).

Island of Molokai.—*Degener* 5,962, a common weed in waste ground, Hawaiian Homes Commission Homesteads, Hoolehua, Apr. 4, 1928 (N.Y.; U.S.).

Island of Lanai.—*Charles N. Forbes* 247*L*, mountains at east end, June, 1913 (Calif.; Kew; N.Y.).

Island of Hawaii.—*Degener* 5,953, on dry, grassy plains, along government road 7 miles west of Kilauea, July 21, 1926 (Gray; N.Y.); *Degener* 5,959, sterile plain, east of Kilauea-Iski, July 16, 1926 (Kew; N.Y.); *Forbes* 164*H*, slopes of Hualalai, June 15, 1911 (Mo.; U.S.); *Joseph F. Rock* 8,319, common weed on Parker Ranch, June, 1910 (Gray, 2 sheets).

[8] The International Rules of Botanical Nomenclature protect a choice so made of course, but there is ever present the possibility that a still earlier author may subsequently be found to have noted the same synonymity but to have chosen the first of the two names for use. It seems unfortunate that such a provision was ever adopted by an international congress of botanists.

[9] *Cf.* Linnaeus (*loc. cit.*), who gave for *S. anglica*, "fructibus reflexis," and for *S. gallica*, "fructibus erectis."



Fig. 2. *Silene Degeneri*, from isotype in Herb. Chicago Nat. Hist. Mus. (photograph by author, his negative no. 3,807).

SCHIEDEA **sarmentosa** Degener & Sherff. sp. nov.—Infra fruticosa et valde ramosa, circ. 3–4.5 dm. alta internodiis subteretibus numerosis saepius ±1 cm. longis, ramis numerosis gracilibus suberec-

tis vel adscendentibus vel interdum humi subprostratis jacentibus striatis et (saltem demum) glabris rursus ramosis, ramulis ultimis gracillimis ac moderate hispidulis. Folia opposita filiformia 1.5–4.5 cm. longa et 0.5–1.5 mm. lata, puberula, marginibus reveluta, 3- vel 5- vernia vel angustissima specie 1-nervia, termino calloso-mucronulata, basi in poculum parvum connata. Inflorescentiae multae (saepe 40–60 pro unica planta), puberulae, plerumque interrupto-thyrsoideae, 1–6 cm. longae et 0.5–1.5 rarius –4 cm. latae, saepe 50–100-florae, ramulis suberectis. Sepala varie ovata vel oblonga vel etiam lanceolata, extus puberula, marginibus plus minusve (praecipue supra medium) scariosa, 2–3 mm. longa, nervis obscuris obsoletisve. Staminodia lineari-oblonga, basi non late dilatata, sepalis dimidio breviora, apice acriter bifida. Stamina demum multum exserta. Capsula ovoideo-conica, demum sepala paulo superans. Semina subnigra, tuberculata, vix 0.5 mm. longa (Fig. 3).

Shrubby and much branched below, about 3–4.5 dm. tall; internodes numerous, subterete, more often ±1 cm. long; branches numerous, delicate, suberect or ascending or at times sprawling, (at least in age) glabrous, again branched, the ultimate branchlets very delicate and moderately hispidulous. Leaves opposite, filiform, 1.5–4.5 cm. long and 0.5–1.5 mm. wide, puberulous, at margins revolute, 3- or 5-nerved or narrowest ones seemingly 1-nerved, at tip callous-mucronulate, at base connate into a tiny cupule. Inflorescences many (often 40–60 to a single plant), puberulous, commonly interrupted-thyrsoid, 1–6 cm. long and 0.5–1.5 or more rarely –4 cm. wide, often 50–100-flowered, branchlets suberect. Sepals variously ovate or oblong or even lanceolate, outwardly puberulous, at edges more or less scarious (especially above middle), 2–3 mm. long, the nerves obscure or obsolete. Staminodia linear-oblong, not widely dilated at base, half as long as sepals, at apex sharply bifid. Stamens finally much exserted. Capsule ovoid-conic, finally somewhat surpassing the sepals. Seeds blackish, tuberculate, scarcely 0.5 mm. long.

Specimens examined: *Otto Degener* 5,947, on very dry, sunny, rocky slope, East Fork of Kawela Gulch, southeastern Molokai, Terr. Hawaii, June 11, 1928 (type, Chi.: isotypes, Berl.; Chi., 2 sheets; Del.; Goth.; Gray; Len.; Mun.; Mus. V.; Par.; Penn.; U.S.; U.V.).

The suite of specimens constituting the basis for this species had been misplaced for several years and so was not available for study and treatment in my recent revision of the genus *Schiedea* (Brittonia 5: 308–336. 1945). In the key there given, this species would go at once to the first letter *c* on page 313. It would fit the first letter *d* thereunder as to the linear-oblong shape of its staminodia, but not as to their tips, which are sharply bifid, not entire. Moreover, its inflorescence, except in atypic cases, tends to be elongate, not more or less globose, and the species is native to Molokai, not Oahu. In general habit the species suggests somewhat *S. spergulina* A. Gray, a plant from the Island of Kauai with 1-nerved leaves. *S. sarmentosa* is perhaps closest to *S. Menziesii* Hook., from which it can be told by its shorter staminodia, these about half as long as (not equalling or somewhat surpassing) the sepals and not widely dilated at base, etc. *S. sarmentosa* perhaps differs most notably from all other species of *Schiedea* in its tendency toward a densely bushy habit, a habit best developed in plants about 3 dm. tall.

Bidens **Ballsii** sp. nov.—Herba sine dubio annua, stricte erecta, ±4.5 dm. alta; caule robusto plus minusve tetragono, glabro vel ad nodos sparsissime pubescenti, internodiis 6–14 cm. longis et usque ad 6 mm. crassis. Folia opposita, tenuiter petiolata petiolis sub 4 cm. longis conduplicate marginatis basim versus interdum hispido-ciliatis alibi glabratis basi connatis; lamina (pricipalium) pinnatim 3–5 (–7)-foliolata, foliolis ovatis vel ovato-lanceolatis subsessilibus serratis utrinque sparsim adpresso-hispidulis lateralibus obtusiusculis circ. 2–2.5 cm. longis terminali acuminato circ. 3.5–4.5 cm. longo. Capitula conferta ramos superiores erectos suberectosve terminantia, ±30 pro unico specimine, radiata, pansa ad anthesin 2–3.2 cm. lata et ±8 mm. alta, pedicellis adpresso-hispidis saepius brevibus. Involucri bracteae exteriores 6–8, lineares, virides, saepe patentes, marginibus aegre basi valde hispidae, 3–6 mm. longae; interiores lanceolato-vel oblongo-ovatae, saturate atropurpureae, paulo longiores, apice puberulentae. Flores ligulati circ. 6 vel 7, aurantiaci; ligula oblongo-obovata circ. 1.1 cm. longa apice subtruncato paulum crenatula. Flores disci numerosi (forsitan 50–60), paleis plus minusve atropurpureis et corollas flavas paulo saepe superantibus, antheris atris vel atropurpureis. Achaenia non visa. Ovaria plana, oblonga, angusto-marginata, glabra, apice calva vel obsolete 2-dentata, sub 1 mm. longa.

An herb, doubtless annual, rigidly erect, ±4.5 dm. tall; stem robust, more or less tetragonal, glabrous or at the nodes very sparsely pubescent, the internodes 6–14 cm. long and up to 6 mm. thick. Leaves opposite, slenderly petiolate; petioles under 4 cm. long, conduplicately margined, toward base at times hispid-ciliate elsewhere glabrate, at base connate; blade of principal leaves pinnately 3–5 (–7)-foliolate, leaflets ovate or ovate-lanceolate, subsessile, serrate, on both surfaces sparsely appressed-hispidulous, lateral ones somewhat obtuse and about 2–2.5 cm. long, terminal one acuminate and about 3.5–4.5 cm. long. Heads crowded together, terminating the upper, erect or suberect branches, ±30 to a single specimen, radiate, 2–3.2 cm. wide when expanded at anthesis and ±8 mm. tall, the appressedly hispid pedicels more often short. Exterior involucral bracts 6–8, linear, green, often spreading, at edges weakly at base strongly hispid, 3–6 mm. long; interior bracts lanceolate-

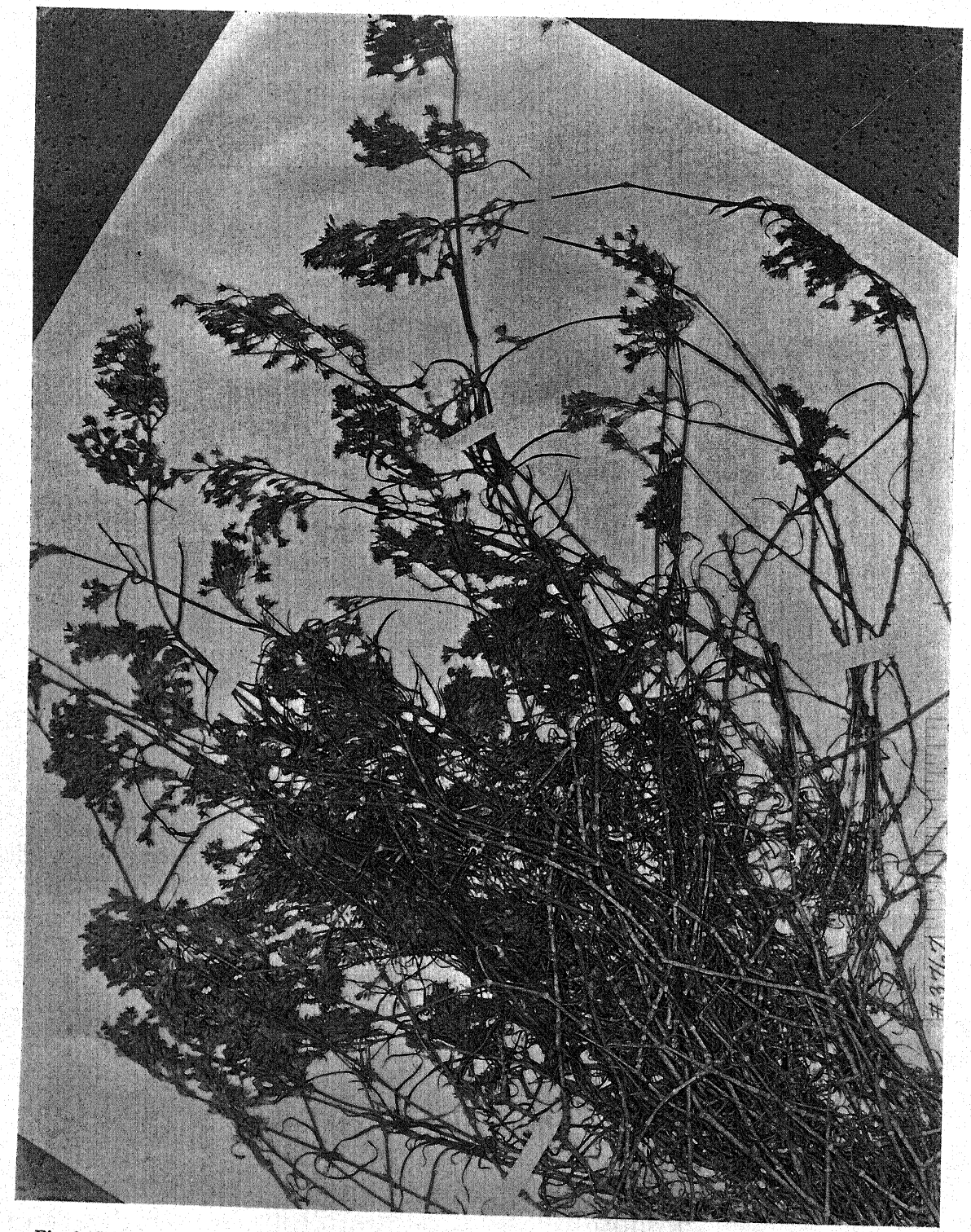


Fig. 3. *Schiedea sarmentosa,* from type sheet in Herb. Chicago Nat. Hist. Mus. (photograph by author, his negative no. 3,767).

or oblong-ovate, intensely dark-purple, a little longer, apically puberulent. Ligulate florets about 6 or 7, orange-colored; ligule oblong-obovate, about 1.1 cm. long, slightly crenulate at the subtruncate apex. Disc florets numerous (perhaps 50–60), the more or less dark-purple paleae often slightly ex-

ceeding their yellow corollas, their anthers black or dark-purple. Achenes not seen. Ovaries flat, oblong, narrow-margined, glabrous, bald or obsoletely 2-dentate at apex, under 1 mm. long.

Specimens examined: *Edward K. Balls* 5,394, in cultivated land, plant strongly aromatic, at altitude of 9,700 feet, Tesmalaquilla, State of Puebla, southern Mexico, August 29, 1938 (type, U.S., herb. no. 1,794,560).

In the key given in my monograph of the genus *Bidens* (Bot. Ser. Field Mus. Nat. Hist. 16, 1937), this species, assuming that its mature achenes are awnless, would belong somewhere under the second *g* of the second *f* of the second *e* on p. 46. Phylogenetically, it would stand between *B. Anthemoides*, p. 491, and *B. serrulata*, p. 526.

The type sheet bears two slightly branched, vigorous, erect specimens, these with most of the heads not yet in flower. These specimens may have been strongly aromatic when fresh, as noted by the collector, but are no longer so.[10]

BIDEN PEDATA Brandegee *ex* M. E. Jones, Contrib. West. Bot. 18: 83. 1935.—In a recent publication ("Asteraceae described from Mexico and the southwestern United States," Contrib. U. S. Nat. Herb. 29: 117–137. 1945), Dr. S. F. Blake has given a valuable interpretation of many ill considered names published by the late Marcus E. Jones. It may be observed here that, in addition to the many names published outright by Jones as new, there were names attributed to other authors but so badly misspelled or distorted as to be essentially different from any names ever published by those authors. Jones' citation of a "*Bidens pedata* Brandegee" appears to be an example of this. He cited *Jones* 27,779 from Laguna, Baja California, as a specimen. I have not seen this cited plant, apparently, but I know of no *Bidens pedata* Brandegee. Doubtless *B. nudata* Brandeg. (Zoë 1: 309, 1890), a species confined to Baja California and collected by Brandegee at Sierra de la Laguna, Oct. 19, 1893 (a specimen seen by me at Kew), was meant.

COSMOS PEUCEDANIFOLIUS Wedd. Chlor. And. 1: 70. 1855.—In my revisional treatment of the genus *Cosmos* (Bot. Ser. Field Mus. 8: 401–447. 1932), southern Bolivia was given (p. 440) as the southern limit for this species. An extension of range southward into the Province of Tucumán, Argentina, is shown by the following three specimens: *C. Bruch* 192, Valle de Tafí, Prov. Tucumán, 1908 (Chi.); *P. Jörgensen* (herb. no.) 35,069, Cerro de Las Paoas, Prov. Tucumán, March, 1911 (Chi.); *S. Venturi* 4,796, alt. 3,600 meters, Estancia Santa Rosa, Dept. Chicligasta, Prov. Tucumán, Jan. 10, 1927 (Chi.).

COSMOS PEUCEDANIFOLIUS var. TIRAQUENSIS (O. Ktze.) Sherff, Bot. Gaz. 88: 307. 1929.—In the revisional treatment just referred to, this variety was similarly listed only for as far south as southern Bolivia. It likewise must now have its range extended southward (though not as far) into Argentina, by the following specimen: *S. Venturi* 7,408, alt. 3,000 meters, Tilcara, Dept. Tilcara, Prov. Jujuy, Feb. 18, 1927 (U.S.).

COSMOS DIVERSIFOLIUS Otto *in* Knowles & Westcott, Flor. Cab. 2: 3, pl. 47. 1838.—In a recent paper (Amer. Jour. Bot. 31: 280. 1944), the range of this species was extended from Mexico into Guatemala as far as Volcán Santa Clara, Department of Sololá. There may be noted here a collection made still further to the southeast in Guatemala, but distributed to herbaria under the wrong determination, *Dahlia Merckii* Lehm.: *Rosalío Gómez* 1,071, alt. 6,500 feet, Santiàgo, Dept. of Zacatepequez, in 1801 (Chi.; Gray; U.S.).

COSMOS DIVERSIFOLIUS var. PUMILUS Sherff. Bot. Gaz. 88: 305. 1929.—Heretofore known only from Boca del Monte, State of Puebla, Mexico. An additional specimen has come to hand from the Delessert Herbarium.[11] Its label bears the number 1,329 and the locality Potosi. In a later hand appears, "Mr. d'Orbigny 1850." Assuming that the Potosi meant is the one in the State of Nuevo Leon, the range of var. *pumilus* is seen to extend considerably to the north or northwest from the locality previously cited.

COSMOS MODESTUS Sherff, Amer. Jour. Bot. 24: 89. 1937.—The type of this rare species (known heretofore from a solitary specimen from Mexico, locality not cited) had been named by Sessé and Mociño, *Coreposis purpurea*, evidently in allusion to the dark-purple ligules.[12] Recently an additional specimen has been examined: *Henri G. Galeotti* 2,028, perennial, rare, woods at 8,000 feet, Cordillera, State of Oaxaca, Mexico, October and November, 1840 (Del.). The collector had labeled the flowers, "pourpre foncé velouté." The dried disk florets of the single well preserved flowering-head are orange-yellow, but the dried ligules are a reddish-black. The dark-purple ligules (which they must have been when fresh) place *C. modestus* definitely next to such species as *Cosmos montanus* Sherff and *C. Scabiosoides* H.B.K. (*cf.* p. 403 in my above cited revision of *Cosmos*), and not with the rosy-rayed *C. diversifolius* Otto, to which

[10] Hillebrand (Fl. Haw. Isls. 211. 1888) noted a "peculiar carroty odor" emitted by various Hawaiian species of *Bidens* (referred by him to a segregated genus, *Campylotheca* Cass.), when they were bruised. This same carroty odor may be obtained, however, by bruising fresh specimens of various North American species of *Bidens*. It seems likely that by "strongly aromatic" the collector of *B. Ballsii* meant something decidedly more than such a carroty odor.

[11] To whose Director, Dr. Charles Baehni, I am much indebted for his great kindness in lending me a large amount of research material.

[12] Sessé and Mociño, as in so many other of their determinations, confused several species or even genera under one name. Thus, a sheet of their *Coreopsis purpurea* at Delessert Herbarium is positively *Dahlia scapigera* (Dietr.) Kn. & Westc.

Hemsley (Biol. Centr.-Amer. 2:199. 1881) once referred Galeotti's plant.

DAHLIA SCAPIGERA **Arsenei** var. nov.—Plus minusve dense pubescens pilis nunc longis multiloculatis mollibusque nunc parvis simplicibus acribus rigidisque. Folia plerumque unipinnata foliolis 3–7 late ovatis vel orbiculato-ovatis, lateralibus utrinque obtusis vel cuneato-rotundatis saepius 1–2 cm. longis latisque, terminali plerumque ovato-cordato ±duplo majore basi truncato cordatove.

More or less densely pubescent with hairs now long, many-celled, and soft; now small, simple, sharp, and rigid. Leaves commonly once-pinnate, leaflets 3–7, broadly ovate or orbiculate-ovate; lateral ones obtuse or cuneate-rotundate at each end, more often 1–2 cm. long and wide; terminal one commonly ovate-cordate, ±twice as large, truncate or cordate at base.

Specimens examined: *Brother George Arsène,* in fields, alt. 2,000 meters, Quinceo, vicinity of Morelia, State of Michoacan, Mexico, June, 1909 (Del.); *Arsène,* slopes, alt. 2,100 meters, vicinity of Morelia, Sept. 30, 1911 (Del.); *Arsène* 18, alt. 2,200 meters, Cerro Azul, vicinity of Morelia, June, 1910 (Chi.; Del.); *Arsène* 5,520, same locality, 1910 (type, Gray: isotypes, Chi.; Mo.; N.Y.; U.S., 2 sheets); *Arsène,* 6,787, alt. 2,200 meters, Campanario, vicinity of Morelia, September, 1911 (U.S.).

DAHLIA BARKERIAE Knowles & Westcott Fl. Cab. 2:28. 1838; 3:147, *tab.* 127. 1840; *Dahlia pubescens* S. Wats., Proc. Amer. Acad. 26:1842. 1891 (*non* Brongn.). The original material of *Dahlia Barkeriae* was collected "in the forests in the vicinity of Valladolid," which we may safely assume was the Valladolid now known as Morelia, in the State of Michoacan, Mexico. Specimens labeled *D. Barkeria* are singularly lacking in American herbaria so far as I have examined. The Index Kewensis (1:710. 1895) reduces the species to *D. variabilis* Desf. (*i.e., D. pinnata* Cav.). However, *D. Barkeriae* differs from that species in its hirsute stems and leaves and in its external involucral bracts, which are of the more conspicuously striate type met with in several species of *Cosmos.* Knowles & Westcott's description and plate were based on cultivated material and the plants were portrayed as especially hairy. Specimens collected later on by Pringle (his no. 3164) in the nearby State of Mexico were referred to *D. pubescens* Wats. They are seen, however, to belong to *D. Barkeriae.* They are pubescent but perhaps even when freshly gathered had fewer and shorter hairs than the cultivated plants used for *D. Barkeriae's* type description and plate.[13] One sheet of the *D. pubescens* Wats. type collection at Gray Herbarium (from the Klatt Herbarium) has a leaf exceedingly similar to the two principal leaves in the type plate of *D. Barkeriae,* but the entire suite of that collection (*Pringle* 3164) shows leaves with leaflets often ranging down to linear or oblong-linear and to subentire. In this last state the resulting foliar appearance is so different from that portrayed in the type plate as to be highly misleading.

Specimens examined: *C. G. Pringle* 3164, calcareous bluffs, Flor de Maria, State of Mexico, Aug. 1 and 28, 1890 (type collection of *D. pubescens* Wats.: Calif.; Chi.; Gray, 2 sheets; Mex., 3 sheets; Mo.; N.Y.; U.S.).

DAHLIA COCCINEA **Palmeri** var. nov.; *Dahlia gracilis nonnull. auct. non* Ortg.—Folia principalia bi- vel tripinnata, segmentis ultimis saepius anguste ad late lanceolatis.

Principal leaves bi- or tripinnate, their ultimate segments more often narrowly to broadly lanceolate.

Specimens examined (all from Mexico): *C. L. Lundell* 5,525, Charcas, State of San Luis Potosi, July and August, 1934 (U.S.); *E. W. Nelson* 4,714, Ramos to Inde, State of Durango, Aug. 11-14, 1898 (U.S.); *Edward Palmer* 494, sheltered recesses of mountains and hills, City of Durango and vicinity, State of Durango, August, 1896 (type, 2 sheets, U.S.: isotypes, Calif.; Chi.; Gray; Mo., 2 sheets; N.Y.); *C. C. Parry & Edw. Palmer* 478, alt. 6,000-8,000 feet, probably in region of San Luis Potosi, 1878 (Gray; Mo.); *C. A. Purpus,* on mesas, El Riego, state not indicated, July, 1905 (Calif.); *Purpus* 3,113, vicinity of San Luis Tultitlanapa, State of Pubela, near Oaxaca, July, 1908 (Calif.; Chi.; Mo.; U.S.); *J. N. Rose* 2,622, road between Mesquitec and Monte Escobedo, State of Jalisco, Aug. 26, 1897 (Gray; U.S., 2 sheets); *Rose, Painter, & Rose* 10,121, near Tehuacan, State of Puebla, Aug. 30-Sept. 8, 1905 (U.S.); *Henry E. Seaton* 357, alt. 8,000 feet, Esperanza, State of Vera Cruz, Aug. 15, 1891 (Chi.; Gray; N.Y.; U.S.); *Forrest Shreve* 9,182, alt. 7,800 feet, mountain slopes south of La Purisima, State of Durango, Aug. 26, 1939 (Gray; rays ±2.6 cm. broad).

Eduard Ortgies received in the summer of 1873 a tuber of a *Dahlia* which Roezl had collected in Mexico. This was planted and flowers were obtained the following summer. Comparing the new plant with the colored plate presented in Sims' treatment of *Dahlia coccinea* Cav. (Curtis's Bot. Mag. 20: 762, *tab.* 762. 1804), he suspected the new plant of representing a new species. The Sims plate showed but one principal leaf, this unipinnate and having 5 leaflets. The Roezl derivative was described as having bipinnate leaves and, after further comparisons, Ortgies named it *Dahlia gracilis* (Regel's Gartenflora *ann.* 1876: 97, *taf.* 861.

[13] The original description of *D. Barkeriae* gave the stems as "very much branched from the base, forming a compact bushy plant . . . 5 to 8 [feet] in circumference." As collected by Pringle, growing spontaneously, the stems were mostly simple below and the circumference would be smaller. There seems no question, however, that the greater amount of branching in the type plants (of *D. Barkeriae*) was due to their having been artificially cultivated (beginning in 1837, at Springfield, near Birmingham, England).

1876). The plate for *D. gracilis* shows foliage more properly designated bipinnatifid, not completely bipinnate. It appears from the accompanying text that Ortgies, who was head gardener at the Botanical Garden in Zurich, slighted Cavanilles' original treatment of *D. coccinea*. Cavanilles (Icon. & Descript. Pl. 3: 32, *tab.* 266. 1795) had raised *D. coccinea* at Madrid and knew its foliage variations. He described the leaves as bipinnate ("foliis bipinnatis") since, as shown in his plate (266), some of the leaves had the lower leaflets again divided ("pinnulis inferioribus iterum pinnatis"). In the Cavanilles herbarium at Madrid, the sheet for *D. coccinea* (neg. no. 29,289 in Field Mus. set of type photographs) has one leaf 2.5 dm. long with seven primary divisions, each of the four lowest being again completely pinnate. *D. coccinea* Cav. is seen, therefore, easily to include within its own normal variations material such as illustrated by Ortgies for his *D. gracilis*.

There is in herbaria today, however, a series of specimens that have the leaves varying from bipinnate to perfectly tripinnate and with the ultimate segments usually narrowly to widely lanceolate, thus narrower and more attenuate-tipped than segments of leaves on *D. coccinea* proper.[14] These specimens have been labeled by some workers as *D. coccinea,* but by others have been construed as typifying *D. gracilis*. They are the ones cited for var. *Palmeri*. They show a geographic range extending from the states of Durango and Jalisco in western Mexico southeastward to the states of San Luis Potosi, Puebla, and Veracruz. For *Dahlia coccinea* proper, the range is greater, extending from the states of Chihuahua, Coahuila, and Nuevo Leon in northern Mexico, southward and eastward through the states of Veracruz, Oaxaca, and Chiapas and into southeastern Guatemala. Except for the more decompound foliage, the var. *Palmeri* displays all of the characters met with in typical *D. coccinea*.[15]

DAHLIA COCCINEA var. STEYERMARKII Sherff, Amer. Jour. Bot. 31: 280. 1944; *Coreopsis crassifolia* Sessé & Mociño, Fl. Mex. edit. 2. 194. 1894; *Dahlia Chisholmi* Rose, Proc. U. S. Nat. Mus. 29: 439. 1905.—In my revision of the genus *Coreopsis* (Bot. Ser. Field Mus. 11: 453. 1936), *Coreopsis crassifolia* Sessé & Moc. was correctly excluded from *Coreopsis* but referred erroneously to *Dahlia variabilis* Desf. The type material had come from mountains in Temascaltepec, State of Mexico. An excellent original specimen, *Sessé, Mociño, Castillo, & Maldonado* 2,926 (Chi.; my photograph no. 3,773) is before me. It agrees closely with and thus well exemplifies the original description of *Coreopsis crassifolia,* except that the outer involucral bracts had been incorrectly described as eight in number ("octophyllus foliolis lanceolatis, reflexis"). They are clearly five on the one complete flowering-head possessed by the specimen. The rays, even after perhaps a century and a half in the herbarium, are of the orange color typical of much of *D. coccinea* material and definitely relate the plant to that species as against *D. variabilis* Desf. (a species more properly reduced to *D. pinnata* Cav.).[16]

Recently there have come to hand for study several additional specimens and suites of specimens that had been collected in the type locality (for *C. crassifolia*) of Temascaltepec District, also in the neighboring State of Guerrero, Mexico and in the somewhat remote departments of Zacapa and Huehuetenango, Guatemala. Three of these (*Chisholm,* near Arcelia, Guerrero) are the original specimens of *Dahlia Chisholmi* Rose and one (*Steyermark* 50,341) is the type of *D. coccinea* var. *Steyermarkii*. An almost unbelievable range of variation is displayed in coarseness of stem- and leaf-habit, in hispidity of stems and leaves, and in amount of dissection of the leaf-blades. Certain material (especially *Steyermark* 29,613, "1st sheet" at Chi.) approaches or matches tripartite-leaved forms of typical *D. coccinea* so thoroughly as to make segregation specifically apart from *D. coccinea* (*cf. D. Chisholmi*) impossible. As a varietal designation under *D. coccinea,* the name *Steyermarkii* must be used, but the original description must be amplified to portray properly the observed variations:

DAHLIA COCCINEA var. STEYERMARKII descript. amplific.—Caules 0.6–3 m. alti, nunc glabrati nunc subvillosi vel dense albido-tomentulosi pilis acribus multiloculatis plus minusve patenti-retrorsis. Folia interdum basim versus adgregata nunc regulariter disposita, valde variabilia, nunc simplicia ac subsessilia vel etiam longo-petiolata (petiolis saepius marginatis alatisve, ±7 cm. longis), lamina ovato-oblonga vel ovali-lanceolata et 1–1.8 dm. longa, apice acuminata basi late cuneata vel subtruncata, submembranacea, glabrata vel (praecipue infra) plus minusve hispida villosave; nunc 3–5 (–7)-partita, foliolis serratis vel diverse et saepe irregulariter 3–partitis cum segmentis basi cuneatis, rhachi plerumque alata. Pedunculi usque ad 4 dm. longi.

[14] For the ready designation of *D. coccinea* in its varietal sense (*i.e.,* the var. α or var. typica as many botanists would term it), we must use, according to the present International Rules, the name **D. coccinea** var. **coccinea** (Willd.) comb. nov.; *Georgina coccinea* var. *coccinea* Willd. Hort. Berol. 2: *t.* 96. 1809 (etiam ex DC. Ann. Par. Mus. ser. 1, 15: 310. 1810).

[15] It may be observed here that many instances occur in certain genera near *Dahlia,* where there is a comparable foliage difference between species and variety (or variety and species). A few examples are: *Bidens reptans* (L.) G. Don and its vars. *Urbanii* (Greenm.) O. E. Schz. and *dissecta* O. E. Schz.; *B. connata* Muhl. and its var. *pinnata* Wats.; *B. riparia* H.B.K. and its var. *refracta* (Brandeg.) O. E. Schz.; *Cosmos ocellatus* Greenm. and its var. *Greenmanii* Sherff; *Megalodonta Beckii* (Torr.) Greene and its var. *oregonensis* Sherff.

[16] Since the above was written, an additional original sheet of *Coreopsis crassifolia* Sessé & Moc. has been received from the Delessert Herbarium for study. This bears two sprays. One is in flower and has simple leaves. The other has the top leaves simple, the others tripartite and very similar to some of those on *Hinton,* 7,999 (Mo.).

Specimens examined: *Ruth Q. Abbott* 147, "2 or 3 meters tall .... flowers various shades of orange —from nearly scarlet to yellow," Taxco (Tasco), Guerrero, July 18, 1936 (Gray); *Frederico* (*Frederick*) *Chisholm* ["Hacienda de Trinidad near Arcelia, Guerrero" *fide inscriptionis novae in herbarii scheda*], 1904 (Sect. of Pl. Introd. no. 9,884, flowered in greenhouse at Washington, D. C., in May, 1905; 2 sheets, U.S., type of *Dahlia Chisholmi* Rose); *Chisholm,* Hacienda de Trinidad near Arcelia, Guerrero, 1904, flowered in greenhouse at Washington, D.C., November, 1904 (U.S., Sect. Pl. Introd. no. 10,573); *George B. Hinton et alii* 7,999, 1.5 meters tall, oak woods on hill, Socabón, Distr. Temascaltepec, State of Mexico, Aug. 17, 1935 (Gray; Mo.; U.S.); *J. N. Rose, Jos. H. Painter, & J. S. Rose* 9,330, in Cañon de la Mano Negra, near Iguala, State of Guerrero, Aug. 11, 1905 (Gray; U.S.; form with broad, ovate leaflets 1.2 dm. long and ±6.5 cm. wide, and with certain simple leaves large, ovate, slenderly petioled, the blade basally rounded-subtruncate); *Sessé, Mociño, Castillo & Maldonado* 2,926, mountains, Distr. Temascaltepec, 1787–1804 (type collection of *Coreopsis crassifolia* Sessé & Moc., Chi.); *Julian A. Steyermark* 29,613, rays orange-vermilion with yellow disk, alt. 1,500–1,700 meters, rocks along ravine, rich forested slopes in deep ravine along Río Lima, Sierra de las Minas, between Río Hondo and summit of mountain at Finca Alejandria, Dept. Zacapa, Guatemala, Oct. 11, 1939 (Chi., 2 sheets, the "2nd sheet" having the four leaves very like those of *Hinton* 7,999 at U.S. but larger [2–2.8 dm. long including petioles, these 5.5–11 cm. long] and with basal leaflets incompletely tripartite, not simple); *Steyermark* 50,341, stem pubescent, 3 feet tall, rays orange, disk yellow, dry, rocky slopes along stream below Calaveras, alt. 2,800–3,700 meters, between Chémal and Calaveras, Sierra de los Cuchumatanes, Dept. Huehuetenango, Guatemala, Aug. 9, 1942 (type, Chi.).

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SIZE-FREQUENCY STUDY OF FOSSIL PINE POLLEN COMPARED WITH HERBARIUM-PRESERVED POLLEN [1]

Murray F. Buell

THE USE of the size-frequency technique in the identification of species of fossil pine pollen introduces a hopeful element into pollen analytical work wherever appreciable quantities of pine pollen are involved. A shift in pine species may have very significant implications. The size-frequency technique applied to the identification of fossil pine pollen was employed by Cain (1940) in the study of pollen-bearing deposits in South Carolina. His papers should be studied for earlier references. This technique is based upon two facts which appear in a study of both modern and fossil pine pollen. First, measurements of the length of a fairly large number of pollen grains of any one species of pine can be plotted in such a way as to produce a curve characteristic in shape and mode position for that species. S. A. and Louise G. Cain (1944) showed that, at least in *Pinus palustris* Mill., there is not a wide variation in the curve from one individual collection to another within the species. Although the pollen of some species produces curves very similar to each other, others yield curves sufficiently distinctive in shape and mode position to serve as a basis for identification. Secondly, Cain found that by plotting measurements of fossil pollen, polymodal curves may sometimes be obtained. In the particular material he was working with the curve happened to be trimodal. The several modes, he suggested, probably represented as many species and their identity could be determined by superposing upon them suitable curves of modern pollen. This necessarily assumes that fossil and modern pollen grains are comparable in size when subjected to the same treatment. In fact, Cain (1944a, p. 135) has remarked that it is unlikely ".... that fossilization shrinks the grains more than does the Erdtman technique of grain preparation." The present paper presents evidence that fossil pollen grains fail to swell to the same extent as similarly treated herbarium-preserved pollen. Furthermore it is shown that the method of preparation used has a very important effect on the size of both modern and fossil pollen.

In the course of a pollen analysis of a large bog, surface samples of peat were examined to determine how the present pine flora of the locality is reflected in the pollen aggregation in peat now forming. The size-frequency curve for this surface material was so far from the expected form that it seemed desirable to determine whether the methods of preparation might be affecting the results or if the process of fossilization might actually have modified the grains.

The bog from which the samples were taken lies just to the east of the hamlet of Jerome in Bladen County, North Carolina. The vegetation of the bog and the immediate vicinity has already been described (Buell, 1946). Most of the bog area is occupied by a forest of *Pinus serotina* Michx. or by a shrub community, throughout which the pine occurs. A long-leaf pine—turkey oak (*Pinus palustris—Quercus catesbaei* Michx.) community described by Wells and Shunk (1931) entirely surrounds the bog on a sandy rim. Beyond the sand margin to the west

[1] Received for publication January 29, 1946.

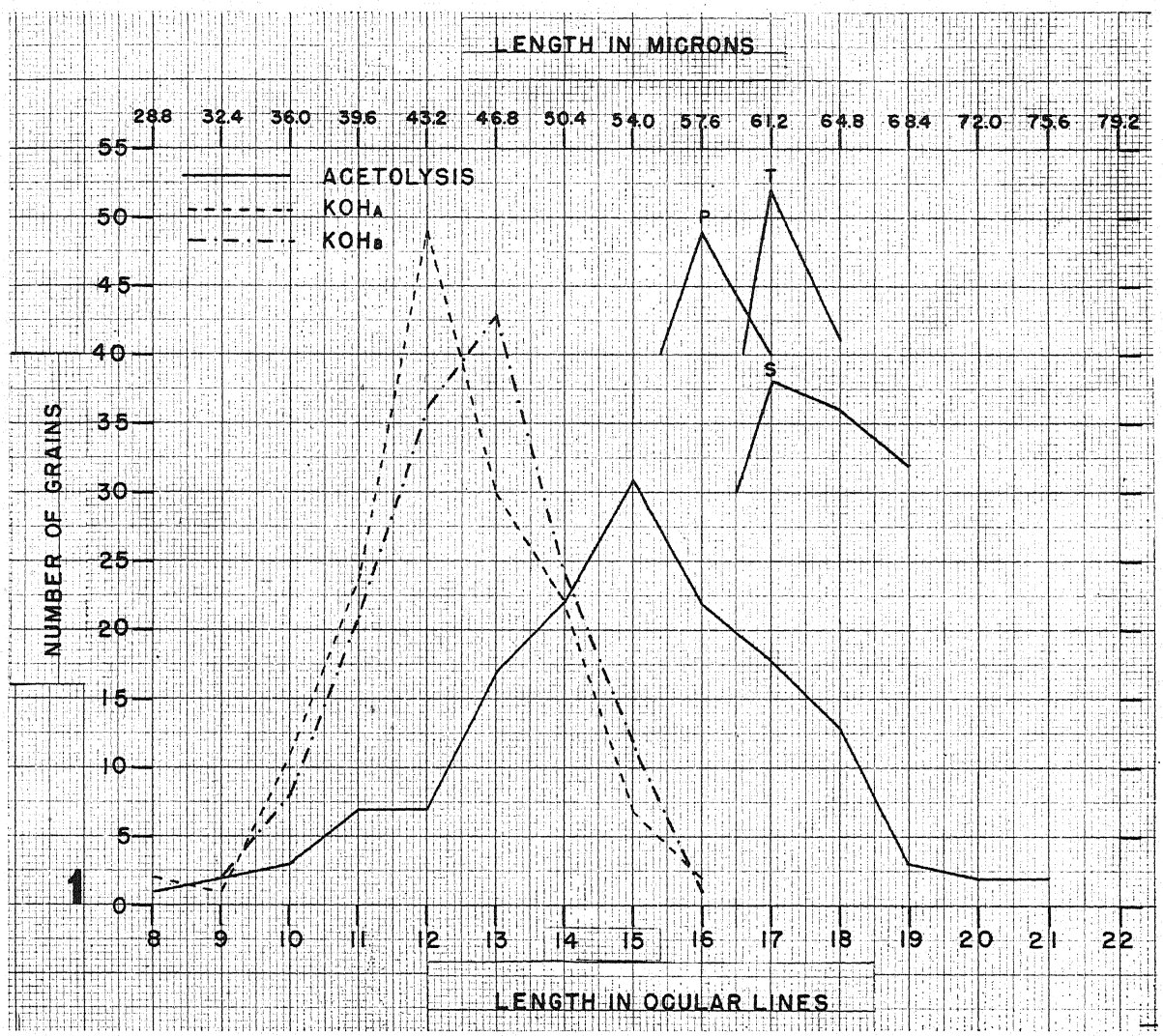


Fig. 1. Size-frequency curves of the pine pollen in one surface sample of peat from the center of Jerome bog. Each curve represents a different method of treatment. The three partial curves in the upper right labeled P, T, and S represent the modes of herbarium *Pinus palustris, P. taeda* and *P. serotina* respectively when treated by acetolysis. They are derived from curves shown in figure 3.

lies a mesic hardwood forest in which occurs *Pinus taeda* L.

An exceedingly large proportion of the pine pollen falling on the center of the bog is very probably derived from trees of *Pinus serotina*. Carroll (1943) has shown that 77 per cent of the pollen collected from bryophytic polsters in spruce-fir forest in the mountains came from the community in which the polsters were obtained. She believes that in flat country the proportion of foreign pollen would be even less. Since Jerome Bog is 4200 by 6500 feet, samples taken near the center are over a quarter of a mile from the margin in any direction. If Carroll's inference is right, it is not improbable that *Pinus serotina* may have contributed as much as 90 per cent or more of the pollen in the surface samples used in the present study.

METHODS.—The peat samples upon which the study is based were obtained by simply brushing aside the most recent fall of leaves and sticks and picking up a handful of surface material reaching to a depth of not more than an inch or two. This material obtained from among the dense mat of rhizomes and roots of the shrubs and trees that occupy the bog is in very early stages of decay. The samples were wrapped in paper and allowed to dry. All collections were made in the winter.

All samples used were taken somewhere near the center of the bog. In fact one sample came from very close to the intersection of the long and the short diameters of the bog. Two came from points on the long diameter 500 and 750 feet respectively southeast of the center. The other three came from points 250, 500, and 750 feet respectively northeast of the

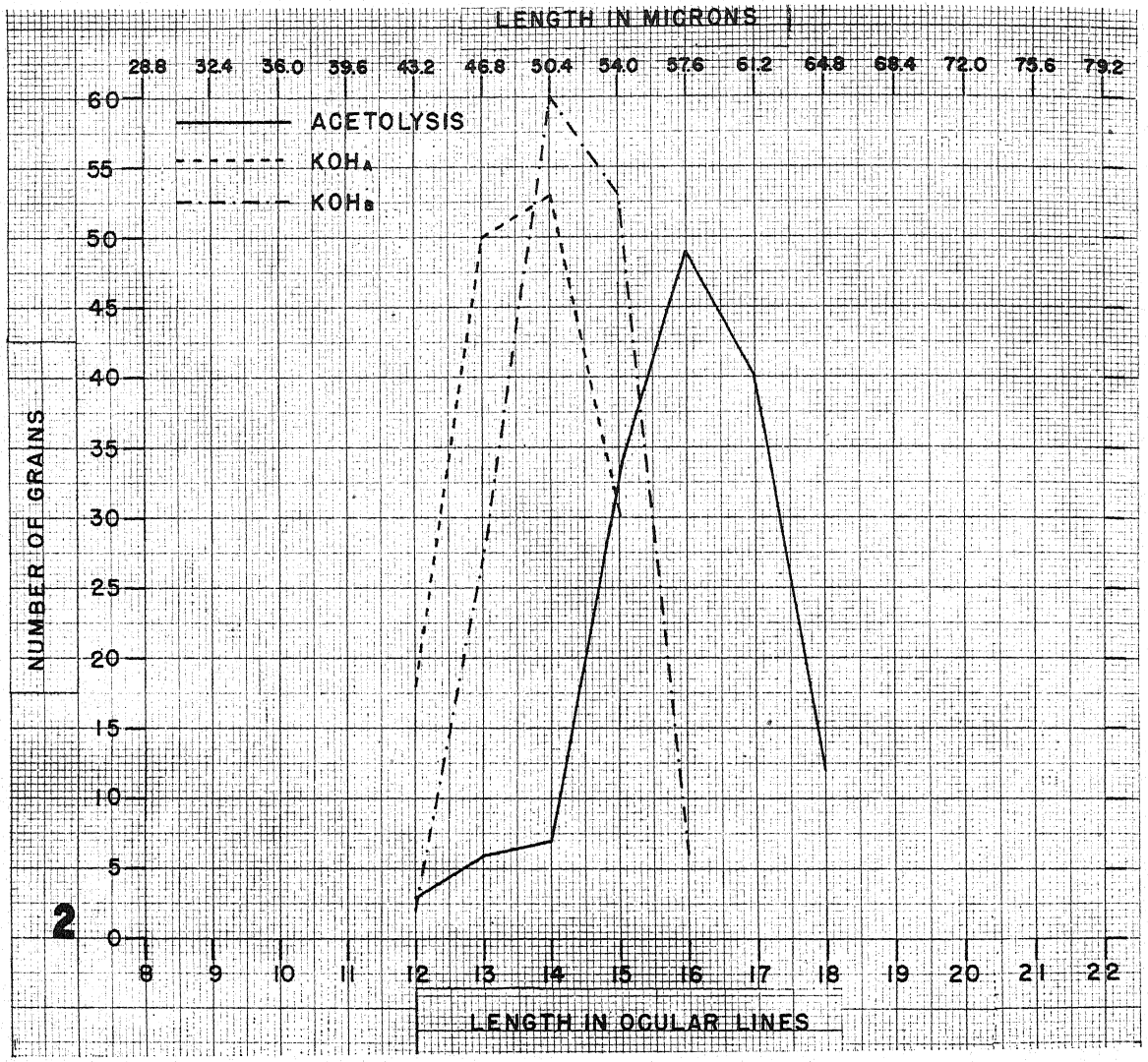


Fig. 2. Size-frequency curves for *Pinus palustris* constructed from pollen prepared by three different methods, KOHa, KOHb and acetolysis as described in the text.

center along the short diameter of the bog. Hence, all collections, although at appreciable distances from one another, were obtained from points more than one quarter of a mile from the edge of the bog.

The reference samples of pollen were all preserved dry, some as herbarium specimens, others in envelopes or vials.

In the comparison of the effect of method of treatment on pollen size three methods were employed, KOH used in two ways and the Erdtman acetolysis method. In one KOH method the peat or the pollen was boiled in a 10 per cent solution of KOH. The boiling was continued about three or four minutes which was long enough to deflocculate the peat and free the grains. This is referred to as the KOHa method in the following text. The second method, referred to hereafter as the KOHb method, involved boiling as described above plus a half hour soaking in the hot KOH allowing the tubes with the mixture to stand in a hot water bath. Finally the acetolysis method was employed as described by its originator (Erdtman, 1943). All material was stained in dilute aqueous safranin and mounted in glycerine gelatin.

In measuring the pollen grains a mechanical stage was used and every grain lying in a suitable position was measured. The dimension used was the internal axis from end to end, considering the bladders to be attached to the ends. This is most commonly, though not invariably, the longest diameter. It is the same as the internal diameter along the line labeled GL in figure 1 as drawn by Cain (1940). Cain used the external diameter in this paper but later in their paper on *Pinus palustris* (1944) he and Louise G. Cain used the internal diameter since they found it so much more uniform and distinct. Only those pollen grains were measured which were lying with this diameter parallel to the plane of the slide.

The same number of pollen grains, 150, was meas-

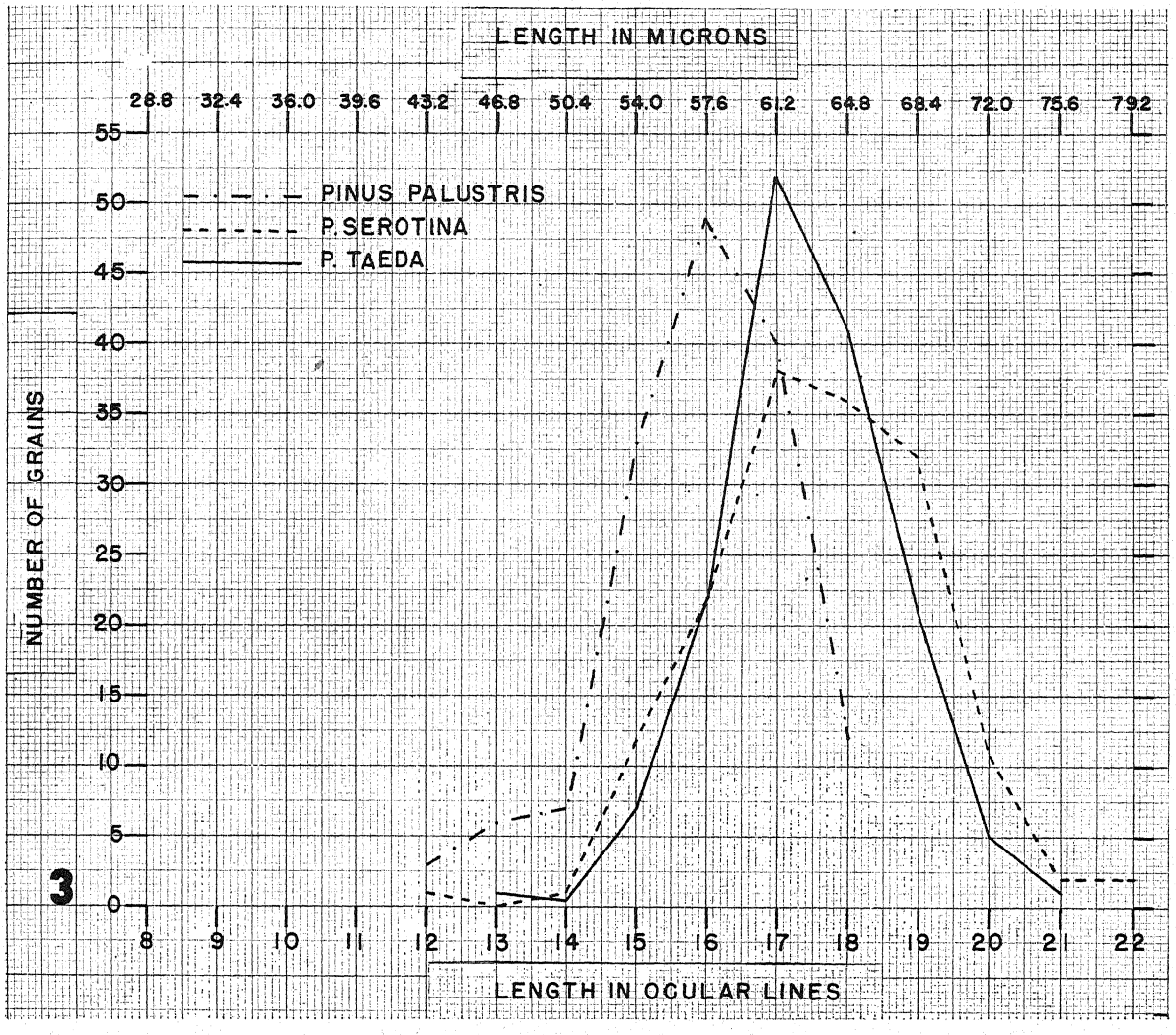


Fig. 3. Size-frequency curves for *Pinus palustris, P. serotina* and *P. taeda* constructed from pollen prepared by the acetolysis method. The *Pinus palustris* curve is based on 150 grains of one collection. Those of *P. taeda* and *P. serotina* are each based on 450 grains, a composite of 150 from each of three collections.

ured on every slide and size frequency curves were constructed on the basis of 150 grains, except where the composite results of several slides were used. When more than 150 grains form the basis of a size frequency curve, it is so indicated in the explanation of the figures.

The units used in tabulating were the lines on the ocular micrometer, the measurement being recorded to the nearest ocular line. The lines were 3.6 microns apart. A magnification of 440 × was used.

Results.—The method of treatment is shown in this study to be of importance when considering the size of the pine pollen. Inspection of the three size frequency curves in figure 1 shows how the methods of treatment used affected the size of fossil grains. The KOHa method yielded a curve with a mode at 43.2 microns (fig. 1, KOHa). The KOHb method yielded a curve with slightly larger grains (fig. 1, KOHb). The acetolysis method, however, produced in general a much more pronounced swelling[2] although a much wider range in size is apparent (fig. 1, acetolysis). In no case did the mode of the size frequency curve of the fossil pollen correspond with that of any species of the reference pollen when prepared in the same way.

Herbarium pollen of *Pinus palustris* was prepared by the same three methods as the peat samples. KOHa produced slightly less swelling than the somewhat longer treatment, KOHb (compare KOHa and KOHb in fig. 2). The acetolysis method, however, caused decidedly greater swelling of the

[2] Although it is not possible to obtain accurate measurements of air-dry and water-soaked pollen such measurements were attempted for the sample of *Pinus palustris* pollen used in preparations for figure 2. Since the air dry pollen was about 7.2 microns smaller and that soaked a month in water was about 3.6 microns smaller than the KOH treated material the term "swelling" is used when referring to the effect of the agents used.

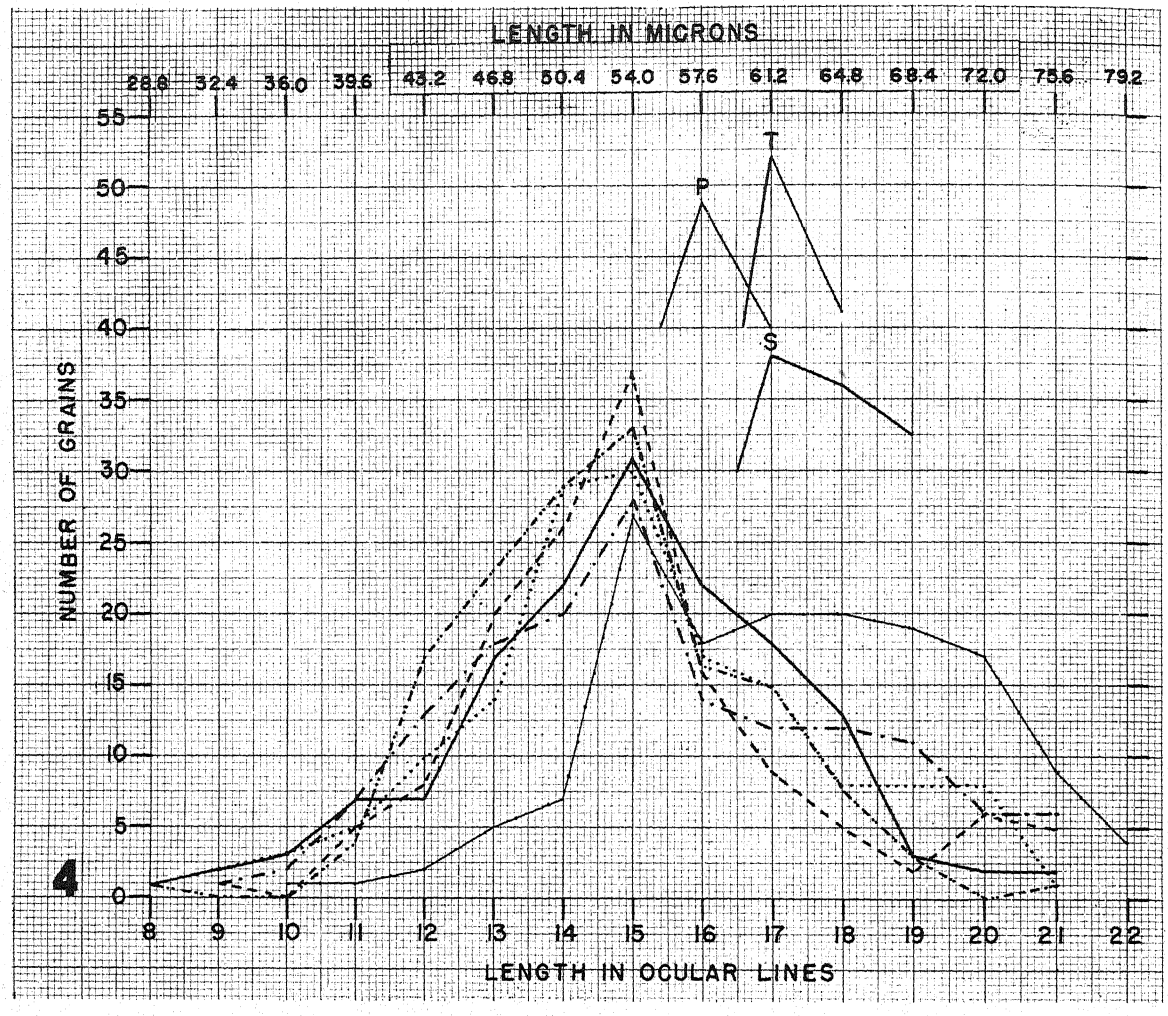


Fig. 4. Size-frequency curves for six surface samples of pollen from Jerome bog. The three partial curves, P. T, and S in the upper right indicate the modes for *Pinus palustris, P. taeda,* and *P. serotina* respectively derived from curves shown in figure 3. All were constructed from material treated by acetolysis.

grains as evidenced by the curve in the same figure. Comparable results were obtained for *Pinus serotina* (table 1).

Reference slides of the three species of pine that could have contributed to the fossil pollen of the surface samples were prepared by acetolysis. The curve obtained for the one sample of *Pinus palustris* with its mode at 57.6 microns (fig. 3) agrees in mode position with three of ten collections studied by the Cains (1944).[3]

The other two species are larger, at least on the basis of the three collections of each that were available. The mode at 61.2 microns is the same for *Pinus taeda* and *P. serotina*. The shapes of the curves differ, however, that of *P. serotina* being somewhat lower and broader than that for *P. taeda* (fig. 3). The shapes of the two curves are strikingly similar to those that Cain (1940) published for the same two species. Size, however, cannot be compared since he was using the outside rather than the inside diameter of the pollen. In constructing the curves of *Pinus taeda* pollen, 150 grains of each of the three collections available were measured. The same is true of the *P. serotina* pollen. The curves of both are hence based on 450 pollen grains. A slight varia-

[3] The composite of their collections would yield a curve with a mode at 54.6 microns but the average pollen size is somewhat greater than this. Thus figure 3 is fairly representative.

TABLE 1. *Size distribution of Pinus serotina pollen from one collection prepared by two different methods: by boiling in 10 per cent KOH (KOHa) and by the acetolysis method.*

	[a] 12	13	14	15	16	17	18	19
	[b] 43.2	46.8	50.4	54.0	57.6	61.2	64.8	68.4
KOHa method ..	0	7	35	80	24	4	0	0
Acetolysis	1	0	5	29	42	47	21	5

[a] Ocular lines. [b] Microns.

TABLE 2. *Summary of measurements of pollen grains on reference slides prepared by acetolysis. Sources of material (pollen not collected in the field by the author was obtained from herbarium specimens of the University of North Carolina collections as indicated): Pinus palustris, Jerome, Bladen Co., N. C.; Pinus taeda, 1, Auburn, Ala., by W. W. Ashe, 4-5-31; 3, Myrtle Beach, S. C., by Radford and Stewart, 4-7-40; Pinus serotina 1, Method, N. C.; 2, Savannah, Ga., by W. C. Coker, 4-18-33; 3, Kinlock, S. C., by W. W. Ashe, 5-1-16.*

	[a] 12	13	14	15	16	17	18	19	20	21	22
	[b] 43.2	46.8	50.4	54.0	57.6	61.2	64.8	68.4	72.0	75.6	79.2
Pinus palustris.....	3	6	7	33	49	40	12	..	..	..	..
Pinus taeda 1.....	..	..	..	8	33	62	33	11	3	..	..
Pinus taeda 2.....	..	..	..	4	14	43	49	31	6	3	..
Pinus taeda 3.....	..	3	1	9	18	51	40	20	7	1	..
Pinus serotina 1.....	1	0	5	29	42	47	21	5	..	..	..
Pinus serotina 2.....	2	0	0	3	14	39	39	34	15	2	2
Pinus serotina 3.....	..	..	..	5	9	28	49	28	8	7	6

[a] Ocular lines. [b] Microns.

tion evidenced in individual collections is shown in table 2.

Six surface samples of peat were treated by the acetolysis method and from slides so prepared size-frequency curves of pine pollen were constructed (fig. 4). All agree in position of the mode at 54.0 microns. On the other hand, the shapes of the curves differ considerably. What they appear to have in common, in addition to the location of the mode, is an especially large number of grains in the larger size brackets.

DISCUSSION.—The different responses of *Pinus palustris* pollen to KOH treatments and acetolysis was not expected. Cain (1944b) had found that *Abies fraseri* (Pursh) Poir. showed practically no difference in size when soaked in hot KOH for twenty minutes and when treated by acetolysis. This indicates that there may be a difference between the reaction of various kinds of pollen to treatments commonly used in separating pollen from peat. It emphasizes the importance of consistently using a uniform method in the preparation of pollen material if size is to be employed as a characteristic for identification.

The failure of fossil pollen to swell to the same extent as herbarium pollen when given the same treatment is suggested in the present study. The size-frequency curves for six surface samples of peat taken from the center of a large bog where about 90 per cent of the pine pollen could be expected to be that of *Pinus serotina* all had a mode 7.2 microns below that obtained from reference slides of *Pinus serotina*. The acetolysis method was used on these samples but it is also shown that KOH would produce similar results (table 1 and fig. 1). It is possible, although it does not seem probable, that the three specimens of *Pinus serotina* collected from widely scattered localities represent individuals with abnormally large grains thus greatly distorting our results. But the Cains (1944) found that only an occasional and perhaps doubtful collection (one out of eleven) of *Pinus palustris* had a mode as much as 6.24 microns away from that produced by most of the other ten. Indeed, that aberrant one they discarded from consideration as possibly incorrectly identified by the collector leaving a modal variation among the rest of less than 4.7 microns. Hence, unless the pollen grains of *Pinus serotina* are exceedingly variable as compared with that of *P. palustris* and the pollen grains from the three samples used in this study were abnormally large it is fairly certain that fossilization, even within a period of a very few years, has an appreciable effect on the ability of pine grains to react to the swelling influence of various treatments. Apparently an important change occurs in the pollen wall very early in the process of fossilization. Likewise there is early disintegration of the protoplasm and reserve foods.

The influence of recent pollen rains would be expected to show up in the curves because of the method of obtaining the surface samples. It does. The asymmetrical curves (fig. 4) obtained from the surface samples reflect a large number of grains larger than the mode which are undoubtedly grains in the early stages of fossilization.

SUMMARY

Treatment of *Pinus palustris* and *P. serotina* pollen with 10 per cent KOH failed to swell the grains as much as the acetolysis method of treatment. Similar differences appeared in the treatment of fossil pine pollen.

Surface samples from the center of a large peat bog indicate that within a very few years pine pollen becomes "fossilized." Such "fossilization" modifies it in such a way that it will not swell to the same size as herbarium pollen of the same species when either KOH or the acetolysis methods are used.

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CHROMOSOME NUMBERS IN POA, AGROPYRON, AND ELYMUS [1]

Marguerite E. Hartung

THE NEED for improving range grasses suited to the varied conditions of the western United States has stimulated much interest in their cytogenetic characteristics. The present investigation is a part of the range grass breeding program being conducted by the Carnegie Institution of Washington in cooperation with the Pacific Coast Division of the Soil Conservation Service.

Emphasis has been placed on members of the genus *Poa* because of their desirability as palatable grasses, their adaptability to a wide range of climatic conditions, their wide variety of forms, and their capacity to reproduce asexually. In particular, the great diversity within this genus offers many possibilities for combining species by hybridization, which has been found to be possible even on an intersectional scale (Clausen, Keck, and Hiesey, 1944, 1945). The usual reproduction of most agronomically important species of *Poa* by apomixis makes probable a selection of F_1 plants that will propagate true to form.

The present survey has been made because information on chromosome numbers, combined with information on geographic distribution of the natural units, is requisite to an understanding of the problems involved. Twenty-six species of *Poa*, represented by approximately 146 races, have been examined, and their somatic chromosome counts are listed in table 1.

TABLE 1. *Poa chromosome numbers.*

Plant number	Species and origin	Elevation in feet	2n
	Poa ampla Merr.		
*4473–11	Susanville, Calif.	4400	64
4171–1	Fort Klamath, Ore. (P-8942)	4200	64
4193–1	Steens Mt., Ore. (P-7743)	....	63
4172–1	Moro, Ore. (P-2716)	ca. 500	63
4173–1	Condon, Ore. (P-5731)	2500	ca. 63
4186–11	Enterprise, Ore. (P-8934)	3500	63
4186–12	Enterprise, Ore., aberrant	3500	ca. 97
4174–1	Wenas, Wash. (P-7820)	3500	63
4175–1	Wenatchee, Wash. (P-837)	685	63
4175–2	Wenatchee, Wash.	685	63
4176–11	Waterville, Wash. (P-999)	2615	62
4177–1	Washtucna dunes, Wash. (P-5728)	1500	63
4178–1	Kahlotus, Wash. (P-846)	900	64
4179–1	Barstow, Wash. (P-8916)	2000	ca. 63
4180–13	Spokane, Wash. (P-4914)	1900	63
4182–11	Colfax, Wash. (P-7772)	1970	63
4183–1	Albion, Wash. (P-8903)	2400	64
4183–13	Albion, Wash., weak aberrant	2400	ca. 100
*4184–1	Clarkston, Wash. (P-6116)	825	63
4185–11	Pomeroy, Wash. (P-6252)	2000	63
4187–11	Cottonwood, Ida. (P-8928)	4000	ca. 65
4196–11	Heise Hot Springs, Ida. (P-6727)	5000	70–71
4199–1	Henry Lake, Ida. (P-6774)	ca. 7000	ca. 63
4593–11	Flathead Co., Mont. (D-310)	....	68
4594–11	Broadus, Mont. (D-806)	ca. 3200	68
4586–11	Medicine Bow Mts., Wyo. (Porter 3308)	9000	64
4188–11	Cheyenne, Wyo. (P-5113)	6100	63
	Poa arachnifera Torr.		
*4434–11	Fargo, Okla. (Harlan), female	ca. 2000	54–56
4434–12	Fargo, Okla., female	ca. 2000	56
4435–12	Woodward, Okla. (Harlan), male	1900	ca. 63
	Poa arida Vasey		
4431–11	Soil Cons. Serv. Nursery, Albuquerque (Harlan)	....	63
4613–11	Glenrock, Wyo. (D-970)	ca. 5000	64
4432–11	Woodward, Okla. (Harlan)	1900	ca. 64
4433–11	Fargo, Okla. (Harlan)	ca. 2000	64
*4262–1	North Platte, Nebr. (P-7936)	2800	63
4262–14	North Platte, Nebr., dwarf	2800	ca. 90
4262–15	North Platte, Nebr., dwarf	2800	ca. 103
4616–11	Belfield, N. D. (D-965)	2600	84
	Poa bulbosa L.		
*4602–11	Dickinson, N. D. (D-1269)	2450	42+f
	Poa Canbyi (Scribn.) Piper		
4224–2	Yosemite Creek, Calif.	ca. 7400	ca. 94
4518–11	Yosemite Creek, Calif.	ca. 7400	93
4225–1	Timberline Station, Mono Co., Calif.	10500	ca. 99
4225–3	Timberline Station, Mono Co., Calif.	10500	ca. 90
4225–5	Timberline Station, Mono Co., Calif.	10500	105–106

[1] Received for publication January 26, 1946.

TABLE 1. *Continued.*

Plant number	Species and origin	Elevation in feet	2n
4230–1	Kiger Creek, Steens Mt., Ore. (P-6003–4)	....	85
4205–1	Chewelah, Wash. (P-8906)	1900	ca. 82
4206–1	Blue Mts., Wash. (P-851)	....	84
4207–1	Mt. Misery, Blue Mts., Wash. (P-2588)	....	84
4209–1	Horse Shoe Bend, Ida. (P-6229)	2700	ca. 82
4210–1	Dubois, Ida. (P-6214)	5000	ca. 83
*4233–1	Emmett, Ida. (P–5980)	2300	84
4211–1	Gallatin Gateway, Mont. (P-6326)	5000	84
4590–11	Pole Mt., Wyo. (Porter 3510)	8200	ca. 72
4595–11	Manyberries, Alberta (D-NE56)	....	ca. 84
	Poa compressa L.		
*4532–1	Crescent Mills, Calif.	3500	42
4606–11	Archer, Wyo. (D-NE146)	6100	42
4607–11	Custer, S. D. (D-906)	5300	42
4260–1	Chorsum, Asia Minor (F.P.I. 109,350)	ca. 4500	50
	Poa confinis Vasey		
*4246–11	Warrenton, Ore. (P-8399), female	10	42
	Poa Cusickii Vasey		
*4531–11	Sierra Valley, Calif.	5000	42
	Poa Douglasii Nees		
*4533–11	Cape Mendocino, Calif.	10	28
	Poa epilis Scribn.		
*4243–1	Steens Mt., Ore. (P–2743)	....	ca. 84
	Poa fibrata Swal.		
4534–11	Squaw Valley, Calif. (Hormay)	5700	64
*4534–12	Squaw Valley, Calif. (Hormay)	5700	63
	Poa glaucifolia Scribn. & Will.		
*4612–11	Glenrock, Wyo. (D-968)	5000	50+f
4614–11	Glenrock, Wyo. (D-971)	5000	50
4611–11	Pilot Knob, Wyo. (Porter 3506)	8500	56
	Poa gracillima Vasey		
4240–13	Fallen Leaf Lake, Calif. (St-3163)	7100	86
4226–1	Timberline Station, Mono Co., Calif.	10500	81
4226–2	Timberline Station, Mono Co., Calif.	10500	ca. 81
4226–3	Timberline Station, Mono Co., Calif.	10500	81
4226–4	Timberline Station, Mono Co., Calif.	10500	ca. 84
4226–5	Timberline Station, Mono Co., Calif.	10500	ca. 84
*4226–6	Timberline Station, Mono Co., Calif.	10500	81

TABLE 1. *Continued.*

Plant number	Species and origin	Elevation in feet	2n
	Poa interior Rydb.		
*4599–11	Medicine Bow Mts., Wyo. (Porter 3309)	9000	28
4600–11	Pole Mt., Wyo. (Porter 3509)	8200	28
	Poa juncifolia Scribn.		
4181–1	Endicott, Wash. (P-998)	1590	63–64
4197–11	Newdale, Ida. (P-6716)	5000	62
*4589–11	Laramie, Wyo. (Porter 3475)	7500	84
*4592–11	Sundance, Wyo. (D-1187)	4750	78
	"Poa longifolia Trin."		
*4245–1	Armenia (F.P.I. 108,531)	....	43
	Poa macrantha Vasey		
*4247–11	Warrenton, Ore. (P-4140)	10	28
	Poa nervosa (Hook.) Vasey		
4255–1	Yosemite Creek, Calif.	7400	63+f?
4255–2	Yosemite Creek, Calif.	7400	63
4255–3	Yosemite Creek, Calif.	7400	63–64
4256–2	Tuolumne Meadows, Calif.	8600	62
4257–1	Timberline Station, Mono Co., Calif.	10200	62–63
4257–2	Timberline Station, Mono Co., Calif.	10200	62
4257–3	Timberline Station, Mono Co., Calif.	10200	63
4257–4	Timberline Station, Mono Co., Calif.	10200	62
4257–5	Timberline Station, Mono Co., Calif.	10200	62
*4257–8	Timberline Station, Mono Co., Calif.	10200	63
4472–2	Ravine Creek, Mono Co., Calif.	10500	70
	Poa nevadensis Vasey		
4475–11	Warren Creek, Mono Co., Calif.	9000	63
4477–3	Westwood, Calif.	5000	ca. 65
4478–2	E. of Westwood, Calif.	5200	63
4534–13	Squaw Valley, Calif. (Hormay)	5700	63
*4192–1	Hart Mt. Pass, Ore. (P-7584)	6000	63
4191–1	Summer Lake, Ore. (P-8941)	4400	ca. 66
4190–1	Seneca, Ore. (P-2646)	ca. 3500	62–63
4189–11	Mitchell, Ore. (P-5755)	2700	64
4194–1	Riddle, Ida. (P-6281)	5600	63
*4200–1	Winchester, Ida. (P-6439)	3600	70
4195–1	Blackfoot, Ida. (P-6349)	4000	63
4198–1	Tetonia, Ida. (P-8804)	6200	63
	Poa palustris L.		
*4601–11	Lloyd, Mont. (D-1066)	ca. 3000	28
4620–11	Harbin, Manchuria (F.P.I. 131,544)	....	28
	Poa pratensis L.		
4436–1	Hume, Fresno Co., Calif.	5500	67
4455–11	Columbia, Calif.	2400	68
4437–11	Groveland, Calif.	3000	50
4252–11	Tuolumne Ranger Station, Calif.	3000	56

TABLE 1. *Continued.*

Plant number	Species and origin	Elevation in feet	2n
4252–17	Tuolumne Ranger Station, subnormal	3000	ca. 80
4253–1	Mather, Calif.	4600	68–69
4253–2	Mather, Calif.	4600	68
4253–3	Mather, Calif.	4600	68
4253–4	Mather, Calif.	4600	68
4253–5	Mather, Calif.	4600	68–69
4253–7	Mather, Calif.	4600	68
4253–8	Mather, Calif.	4600	68
4253–9	Mather, Calif.	4600	49
4254–1	Cottonwood Mdw., Tuolumne Co., Calif.	6000	68
4254–3	Cottonwood Mdw., Tuolumne Co., Calif.	6000	67
4254–4	Cottonwood Mdw., Tuolumne Co., Calif.	6000	67
4254–5	Cottonwood Mdw., Tuolumne Co., Calif.	6000	73
4254–6	Cottonwood Mdw., Tuolumne Co., Calif.	6000	67
4444–11	Crane Flat, Tuolumne Co., Calif.	6200	ca. 56
4445–6	Timberline Station, Mono Co., Calif.	10000	ca. 73
4445–8	Timberline Station, Mono Co., Calif.	10000	74–77
4446–13	Warren Creek, Mono Co., Calif.	9000	ca. 68
4446–12	Warren Creek, Mono Co., Calif., dwarfish alpine type	9000	76
4447–11	Below Warren Creek	8700	54
*4258–1	Leevining Creek, Calif.	7350	68
4258–2	Leevining Creek, Calif.	7350	68+f
4258–3	Leevining Creek, Calif.	7350	68
4259–1	S. of Leevining, Calif.	6800	64
*4259–2	S. of Leevining, Calif.	6800	49
4259–3	S. of Leevining, Calif.	6800	68
4453–11	Tioga Lodge, Mono Co., Calif., dry, pumice slope	6500	50
4458–11	W. of Sierra City, Calif.	3600	56
4462–1	Westwood, Calif.	5000	58
4465–1	Blodgett, Ore.	ca. 500	ca. 70
4465–11	E. of Corvallis, Ore.	225	67
4639–11	Logan, Utah (W-X82)	4500	52
4638–11	Montrose, Colo. (W-313-43)	5790	ca. 56
4636–11	Kansas State 3939	....	50
4637–11	Kansas State 3950	....	49–50
4608–11	Mandan, N. D. (D-NE145)	1600	68
4249–1	Athabaska, Canada ("Delta strain")	....	70
4621–11	Valdres, Norway (W-Löken No. 2)	....	70
4621–12	Valdres, Norway	....	66
4623–11	Svalöv, Sweden (W-P. 5)	....	54
4623–12	Svalöv, Sweden	....	50–51
4624–11	Landskrona, Sweden (W-"Weibulls Primo")	....	76
4628–11	Turkey (F.P.I. 119,684)	....	ca. 66
4618–11	Steppes, Manchuria (F.P.I. 131,541)	....	64
4619–11	Mountain district, Manchuria (F.P.I. 131,543)	....	56
4617–11	(W-F.I.-39-74P)	....	78

TABLE 1. *Continued.*

Plant number	Species and origin	Elevation in feet	2n
	Poa pratensis, maritime ecotype		
4466–1	Newport, Ore.	25	81
*4467–11	Boiler Bay, Ore.	25	84
	Poa pratensis ssp. *alpigena* E. Fries		
4250–1	Near Abisko, Swedish Lapland	1400	ca. 74
4250–3	Near Abisko, Swedish Lapland	1400	ca. 68
*4250–4	Near Abisko, Swedish Lapland	1400	76
4250–5	Near Abisko, Swedish Lapland	1400	ca. 73
	Poa rhizomata Hitchc.		
*4609–11	E. of Hamburg, Siskiyou Co., Calif. (St-3263)	1700	28
	Poa scabrella (Thurb.) Benth.		
*4495–1	Gorman, Los Angeles Co., Calif.	3700	44+f
4221–11	Monolith, Calif. (P-12509)	4000	84
4212–4	Las Posas, Ventura Co., Calif. (P-11040)	575	82
4212–7	Las Posas, Ventura Co., Calif.	575	84+f
4484–11	Las Posas, Ventura Co., Calif.	575	81
4213–11	King City, Calif. (P-11789)	400	86
4214–1	Watsonville, Calif. (P-10099)	100	82
4214–4	Watsonville, Calif., female	100	ca. 62
4215–11	Mt. Hamilton, Calif. (P-12529)	2800	84
4490–11	Clear Lake Highlands, Lake Co., Calif.	1640	84
4217–11	Lucerne, Lake Co., Calif. (P-12337)	1350	ca. 68
4217–16	Lucerne, Lake Co., Calif.	1350	64
*4217–17	Lucerne, Lake Co., Calif.	1350	63
4217–18	Lucerne, Lake Co., Calif.	1350	63
4218–11	Sherwood Valley, Mendocino Co., Calif. (P-12341)	....	84
4219–11	Sherwood Valley (P-12343)	....	63
4511–11	Wildwood, Trinity Co., Calif.	3400	ca. 84
4510–11	Platina, Shasta Co., Calif.	2900	61–63
4530–11	Beegum, Shasta Co., Calif.	2400	104
4229–11	Red Bluff, Calif. (P-11891)	400	84
*4228–11	Anderson, Calif. (P-12716)	600	84
4509–11	Quincy, Calif.	3500	ca. 88
4223–11	NE. of Mather, Calif.	4800	84
4223–12	NE. of Mather, Calif.	4800	84
4223–13	NE. of Mather, Calif.	4800	63
4223–14	NE. of Mather, Calif.	4800	81
4223–15	NE. of Mather, Calif.	4800	84
4223–17	NE. of Mather, Calif.	4800	ca. 91
4222–2	Mather, Calif.	4600	84
	Poa secunda Presl		
4231–11	W. of Fossil, Ore. (P-6002)	ca. 2600	86
4239–1	Pilot Rock, Ore. (P-6005)	2500	ca. 74
4234–1	Mayfield, Ida. (P-2731)	ca. 2700	ca. 87
*4235–1	Duck Valley Indian Reserv., Ida. (P-6274)	5400	84
4236–1	American Falls, Ida. (P-6209)	4350	81
4237–1	Dillon, Mont. (P-5881)	5100	85–87

TABLE 1. *Continued.*

Plant number	Species and origin	Elevation in feet	2n
	Poa sphondylodes Trin.		
4603–11	Naran Obo, China (F.P.I. 113,218)	....	28
*4604–11	Darkhan, Wang Fu, China (F.P.I. 113,018)	....	28
	Poa subfastigiata Trin.		
4605–11	Manchuria (F.P.I. 131,543)	....	91–92+ff.
*4605–12	Manchuria	....	97

Data were obtained on species of *Agropyron* and *Elymus* that were grown in the garden for possible future investigations, and chromosome numbers of these are given in tables 2, 3, and 4.

The author wishes to express her sincere appreciation to Dr. Jens Clausen and Dr. W. M. Hiesey for their helpful advice and assistance in this work, and to Dr. D. D. Keck for his valuable aid in the preparation of the illustrations, species determinations, and the organization of the material. She is indebted to all three for their constructive criticism of the manuscript.

METHODS.—Some bud fixations were made, but it was found that pollen fertility in *Poa* varies considerably within the species, making difficult the securing of uniformly good material for meiotic study. Furthermore, meiosis in most of the species with high chromosome numbers is very irregular, and it is not possible to make accurate interpretations, because the chromosomes are crowded in the pollen mother cells. Somatic material was found to be more dependable, and root-tip divisions were used in determining the chromosome numbers.

TABLE 2. *Agropyron chromosome numbers.*

Species and origin	2n
Agropyron caninum (L.) Beauv.	
Alma-Ata, Turkestan, U.S.S.R. (F.P.I. 107,868)	28
F.P.I. 133,138	28
A. cristatum (L.) Gaertn.	
Fairway variety (P-3171)	14
Lincoln, Nebr. (P-3063)	28
Middle Volga Region, U.S.S.R. (F.P.I. 108,421)	28
Eskishehir, Turkey (F.P.I. 109,218)	28
Ankara, Turkey (F.P.I. 109,012)	28
A. dasystachyum (Hook.) Scribn.	
The Dalles, Ore. (P-1822)	28
Pocatello, Ida. (P-6291)	28
A. Elmeri Scribn.	
The Dalles, Ore. (P-1824)	28
A. elongatum (Host) Beauv.	
Maikop, Caucasus, U.S.S.R. (F.P.I. 98,526)	70
*Troy, Turkey (F.P.I. 119,603)	70
A. inerme (Scribn. & Sm.) Rydb. (See table 3).	
A. intermedium (Host) Beauv.	
Maikop, Caucasus, U.S.S.R. (F.P.I. 98,568)	42
Bolu, Turkey (F.P.I. 109,219)	43
A. Michnoi Roshev.	
Mongolia (F.P.I. 113,039)	28
A. pauciflorum (Schwein.) Hitchc.	
San Benito, Calif. (St-2776)	28

TABLE 2. *Continued.*

Species and origin	2n
Leesville, Calif. (St-2957)	28
Mather, Calif. (7 plants)	28
Yosemite Creek, Calif. (3 plants)	28
Tuolumne Meadows, Calif.	28
Timberline Station, Mono Co., Calif. (5 plants)	28
Warren Creek, Mono Co., Calif. (2 plants)	28
Leevining Creek, Calif.	28
Little Last Chance Valley, Plumas Co., Calif. (St-2926)	28
Lake Co., Ore. (P-876)	28
Goldendale, Wash. (P-3971)	28
Prosser, Wash. (P-7238)	28
"Pacific Northwest" (P-1914)	28
Greenleaf, Ida. (P-9115)	28
Beebe, Mont. (P-2535)	28
Colorado Springs, Colo. (P-5365)	28
Saskatchewan, Canada (Mecca strain)	28
A. Pringlei (Scribn. & Sm.) Hitchc.	
Glen Alpine, Calif. (St-3310)	28
A. riparium Scribn. & Sm.	
Grant Co., Ore. (P-2415)	28
*Pole Mt., Wyo. (Porter 3507)	28
A. sibiricum (Willd.) Beauv.	
Kasakstan, U.S.S.R. (F.P.I. 108,434)	28
Chelkar, U.S.S.R. (F.P.I. 108,319)	28
A. Smithii Rydb.	
Plush, Ore. (P-9373)	28
*Burke, Wash. (P-727)	56
Howe, Ida. (P-6397)	56
Rosebud, S. D. (P-2787)	56
SE. Kansas (P-3194)	56
Perrington, Tex. (P-6887)	56
A. spicatum (Pursh) Scribn. & Sm. (See table 3).	
A. subsecundum (Link) Hitchc.	
Union, Ore. (P-3966)	28
Molson, Wash. (P-931)	28
Plaza, Wash. (P-7510)	28
Pocatello, Ida. (P-6294)	28
Cheyenne, Wyo. (P-4696)	28
A. tauri Boiss. & Bal.	
*N. of Malatya, Turkey (F.P.I. 109,607)	28
A. trichophorum (Link) Richt.	
*F.P.I. 107,330	42

Thirty or more individuals of each race have been grown for study in the gardens of the Carnegie Institution of Washington at Stanford University. Fixation was made of roots of one or more plants of each race before they were set out in the garden. They were grown in sterilized soil in 3-inch pots, but often an inadequate number of good roots was obtained. Therefore, efforts were made to improve the quantity and quality of roots by growing a few strains in water culture, sand, and moss. Hoagland's culture solution was used. The roots in the water and moss cultures were cleaner and better shaped, but little or no improvement was observed in the number and quality of mitotic figures. It is

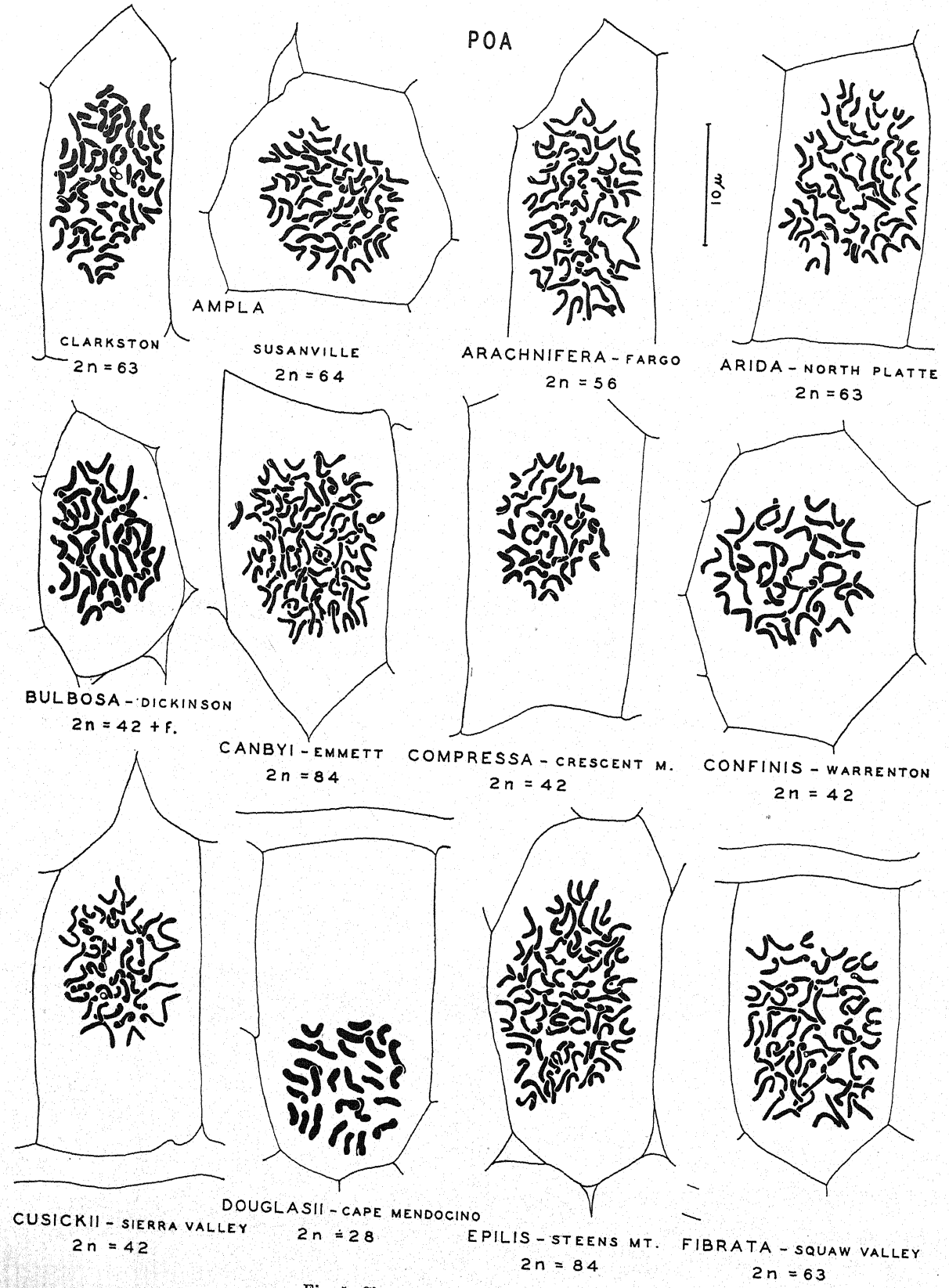


Fig. 1. Chromosomes of species of *Poa*.

TABLE 3. *Agropyron spicatum chromosome numbers, with the number of spicatum and inerme individuals in the 19 cytologically investigated populations.*

Origin of race	Number of individuals: spicatum	inerme
Diploids, 2n = 14:		
Susanville, Calif.	8	..
Canby, Calif. (P-6078)	28	..
Shaniko, Ore. (P-2719)	23	3
Mabton, Wash. (P-738)	19	5
Walla Walla, Wash. (P-9180)	27	2
Lind, Wash. (P-7413)	..	28
Soap Lake, Wash. (P-6018)	..	18
Coulee City, Wash. (P-742)	..	25
Spokane, Wash. (P-4915)	..	30
*Oneyka, Wash. (P-6411)	..	25
Colton, Wash. (P-3537)	..	20
Pullman, Wash. (P-2522)	..	25
Heise, Ida. (P-2724)	29	..
Osgood Range, Nev.	both present	
Tetraploids, 2n = 28:		
Blue Mts., Wash. (P-739)	17	2
Soap Lake, Wash. (P-6025)	28	..
Joel, Ida. (P-737)	24	5
Lewiston, Ida. (P-6409)	28	..
Winchester, Ida. (P-7845)	25	3

probable, though, that the desired results could be obtained by modifying the culture solution.

Müntzing's (1933) modification of Navaschin's solution was used for the killing and fixing process. The *Agropyron* and *Elymus* material presented no special problems, but the *Poa* roots have a thick cuticle that hardens during processing and makes cutting difficult. Most of the tearing of sections was eliminated by soaking the paraffin blocks in water overnight. Sections were cut 12 or 14 microns thick.

Poa chromosomes are usually numerous and fairly small. Crowding and overlapping in the metaphase plates makes accurate counting difficult, especially when the stain is not well differentiated. A satisfactory modification of the crystal violet technique was obtained from Dr. D. A. Johansen, who noted it in Emig (1941). This consists in the addition of 5 ml. of a 5 per cent aqueous solution of ammonia alum and 0.5 ml. of glacial acetic acid to 95 ml. of 0.1 per cent aqueous crystal violet solution. Although the chromosomes do not stain very densely in this mixture, better differentiation is obtained. The preparations fade rapidly and should be examined within two months after being made.

Counts were always made from drawings of metaphase plates, either made freehand or with the aid of a camera lucida. All the plates illustrated were made with the aid of a camera lucida at an original magnification of ×3200.

Owing to the nature of the *Poa* material, individual interpretations of the metaphase plates may result in slight differences in the number of chromosomes counted in all but the clearest figures. This fact shoould be taken into consideration when judging the determinations. Those figures which are admittedly uncertain by a margin of one or two chromosomes are indicated by the prefix *"ca."*

The illustrations of *Poa* (fig. 1–3) are arranged alphabetically by species, excepting in fig. 2 where the maritime ecotype of *pratensis* precedes *juncifolia*. The same arrangement is followed in the tables, with the races listed geographically from south to north and west to east. The names employed are in accord with the treatment in Hitchcock's Manual (1935), even though current cytogenetic investigations call for some revision in it. Those plants whose chromosomes are illustrated are designated in the tables by an asterisk.

MATERIALS.—The grasses used in this investigation were mainly from collections made by the Soil Conservation Service and the Carnegie Institution of Washington. Some species were contributed by Dr. G. L. Stebbins, Jr., of the University of Cali-

TABLE 4. *Elymus chromosome numbers.*

Species and origin	2n
Elymus canadensis L. Washington: Tonasket (P-6684); Wenatchee (P-786); Sunnyside (P-7840). Oregon: Ontario (P-7540). Idaho: Spaulding (P-6608); Idaho Falls (P-2623). Utah: Tremonton (P-9038); Salt Lake City (P-7690). Montana: Bozeman (P-5774). Colorado: Colorado Springs (P-4678). New Mexico: Lincoln Co. (P-4669). Kansas: Hays (P-4673); Atlanta (P-8173).	28
E. cinereus Scribn. & Merr. California: Mono Inn (4 plants).	28
E. glaucus Buckl. California: Santa Paula (St-2865); San Benito (St-2777); Sargent (St-2752); Emerald Lake, San Mateo Co. (4 plants); Cedar Mt., Alameda Co. (St-2725); Wildcat Canyon, Contra Costa Co. (St-3343 and 3345); Bodega Bay (St); *Monte Rio; Hough Springs (St-2960); Bartlett Mt. (St-2966); Garberville; Mattole River; Priest Grade, Tuolumne Co.; Groveland; Tuolumne Ranger Station; Mather (4 plants); Cottonwood Meadow, Tuolumne Co.; Yosemite Creek (2 plants); Timberline Station, Mono Co.; Auburn; Meadow Valley (St-2905); Lake Almanor; Paynes Creek (St-2949). Oregon: Steens Mt. (P-5871); Crater Lake (P-4733); Warrenton (P-5460 and P-8380); Vernonia Junction (P-8992); Union (P-1753). Washington: Dayton (P-3334); Wenatchee Natl. Forest (P-2662); Mt. Spokane (P-3750). Idaho: Rocky Point (P-6626); Moscow Mts. (P-792); Big Falls (P-8826).	28
E. junceus Fisch. *Middle Volga Region, U.S.S.R. (F.P.I. 108,494).	14
E. triticoides Buckl. California: Stanford University; Tuolumne Ranger Station; Leevining; Mono Inn. Oregon: Plush (P-4736), awned; Hot Lake (P-7446). Washington: Sunnyside (P-4961). Idaho: SE. of Boise (P-2741).	28
E. virescens Piper. California: Bodega Bay (St-3366).	28

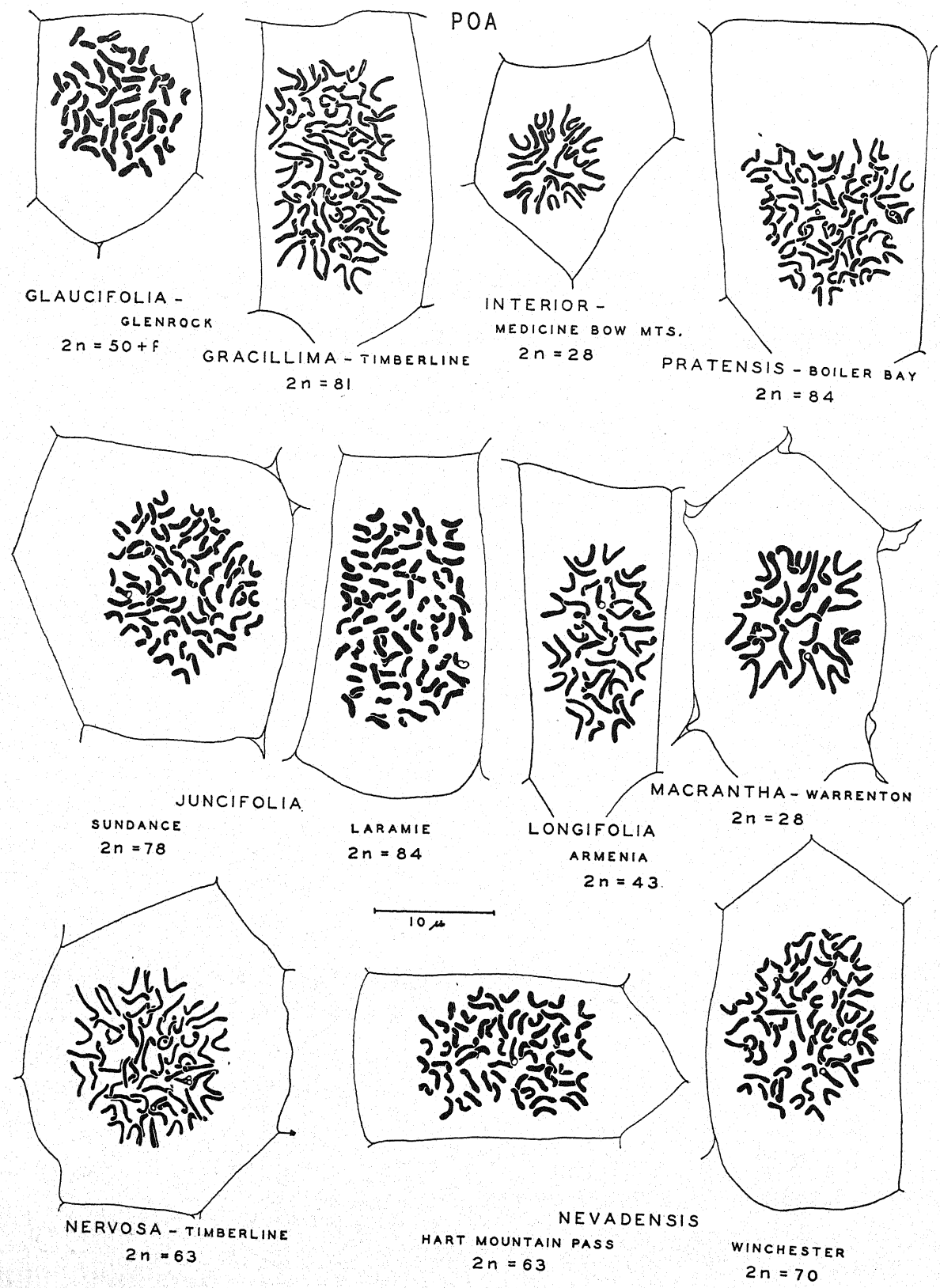


Fig. 2. Chromosomes of species of *Poa*.

fornia, by the Division of Forage Crops and Diseases of the Bureau of Plant Industry, and by others.

In the tables the plant number is that assigned in the gardens of the Carnegie Institution. It consists of the four-digit culture numbers followed by the subnumber of the individual. Parenthetical numbers or symbols following the place name are those under which materials were received from other sources. The key to them is as follows:

D = Northern Great Plains Field Station, Mandan, N. D.
F.P.I. = Foreign Plant Introduction, U. S. Department of Agriculture.
Harlan = Dr. J. Harlan, Southern Great Plains Field Station, Woodward, Okla.
Hormay = Mr. A. L. Hormay, California Forest and Range Experiment Station, University of California, Berkeley.
P = Pacific Coast Division of the Soil Conservation Service.
Porter = Dr. C. L. Porter, University of Wyoming, Laramie.
St = Dr. G. L. Stebbins, Jr., University of California, Berkeley.
W = Dr. E. L. Nielsen, Division of Forage Crops and Diseases, U. S. Department of Agriculture, University of Wisconsin, Madison.

Species of Poa.—The genus *Poa* is worldwide in its distribution and some of the species are very important agricultural grasses. Throughout the United States its members are common constituents of meadows and cultivated pastures, while some are among the most important range grasses of the West. The majority of those represented in this paper can be divided into two main groups: (1) the western American bunchgrasses, referred by Hitchcock to the sections *Nevadenses* and *Scabrellae,* and (2) the rhizomatous or sod-forming members of the section *Pratenses.* Other species studied belong to the sections *Epiles* and *Palustres,* or are of foreign introduction and miscellaneous affinity.

The principal bluegrasses of the western ranges belong to the first group. *Poa ampla* is an outstanding bluegrass of the prairies of the Pacific Northwest, being of particular value to stockmen throughout western Idaho and eastern Washington and Oregon. The typical form is tall and vigorous, with foliage more or less glaucous. *Poa nevadensis* covers much the same territory as *ampla,* but grows at higher elevations and in moister, more meadowy habitats. It is distinguished from *ampla* by its long, pointed ligule, and its softer, usually greener herbage. Both have $2n = 63$ chromosomes (fig. 1–2), with aneuploid and euploid variations from the 7-series occurring occasionally in *ampla* and frequently in *nevadensis.* These results are in agreement with those of Armstrong (1937), who reported counts of $2n = 64$ for two plants of *ampla* and of $2n = 62$ for two of *nevadensis.* Stebbins and Love (1941) gave an approximate $2n$ count of 64–66 for *nevadensis.*

Poa juncifolia, an alkaline meadow grass, is a third member of the *Nevadenses.* It has many of the characteristics of *ampla,* including the short ligule, and the two are distinguished with sufficient difficulty as to raise the question of the specific validity of the two units. Forms that on account of their involute leaves would be classified as *juncifolia* vary considerably in chromosome number (fig. 2 and table 1).

Poa scabrella, secunda, gracillima, and *Canbyi* compose the section *Scabrellae.* They are a group of closely related and intergrading species from low, medium, and high altitudes. All are spring-flowering and, with the exception of *gracillima,* strictly summer-dormant, in contrast with the *Nevadenses* whose members are partially summer-active. *Poa scabrella* is the only bunchgrass *Poa* at low elevations in cismontane California, where it is a valuable range grass in the Coast Ranges and Sierran foothills. *Poa secunda* is the most slender and ephemeral of these bunchgrasses, going dormant relatively early in the season and occurring in typical form across the plains of the Columbia and Snake, well east of the Cascades, at relatively low altitudes. *Poa gracillima* is the high montane counterpart of *secunda.* It has open panicles and, unlike *secunda,* remains summer-active when moved to lower altitudes.

All three, *Poa scabrella, secunda,* and *gracillima,* have a somatic chromosome number usually near the duodecaploid level of $2n = 84$ (fig. 3), although nonaploid individuals ($2n = 63$) have been found sporadically in several populations. In the case of *Poa scabrella* from Lucerne, however, the entire population appears to be nonaploid (fig. 3), but another race from Clear Lake Highlands nearby has the normal number of 84 chromosomes. The Lucerne race differs in appearance from the other Coast Range forms of that species and flowers 6 to 8 weeks later, but is probably a local variant. Likewise, an approximately hexaploid plant of *scabrella,* with $2n = 44 + 1$ fragment, came from Gorman (fig. 3) on the western arm of the Mohave Desert. This plant, which occurred in a typical desert plateau association, was morphologically a true *scabrella* but bloomed later than the coastal forms of the species. A race from Monolith nearby had 84 chromosomes, the typical number for *scabrella,* but its classification is a trifle uncertain since the plants died at Stanford before they could flower. The hexaploid type is therefore probably only a local form. Under *Poa scabrella,* Stebbins and Love (1941) report $2n$ numbers of 63, *ca.* 66, 84, and 86. Those given under *"secunda"* in the same paper, $2n = 82$, *ca.* 84, and 86, are probably also to be referred here, because those plants came from within the range of *scabrella* rather than *secunda.* Armstrong (1937) gives two counts of $2n = 84$ for *scabrella.*

Poa Canbyi, the fourth member of the *Scabrellae,* is primarily a Great Basin species to which certain forms from the Sierra Nevada are now referred. It grows in the same territory as *secunda* and *gracillima* but is found on deeper soils; it is more

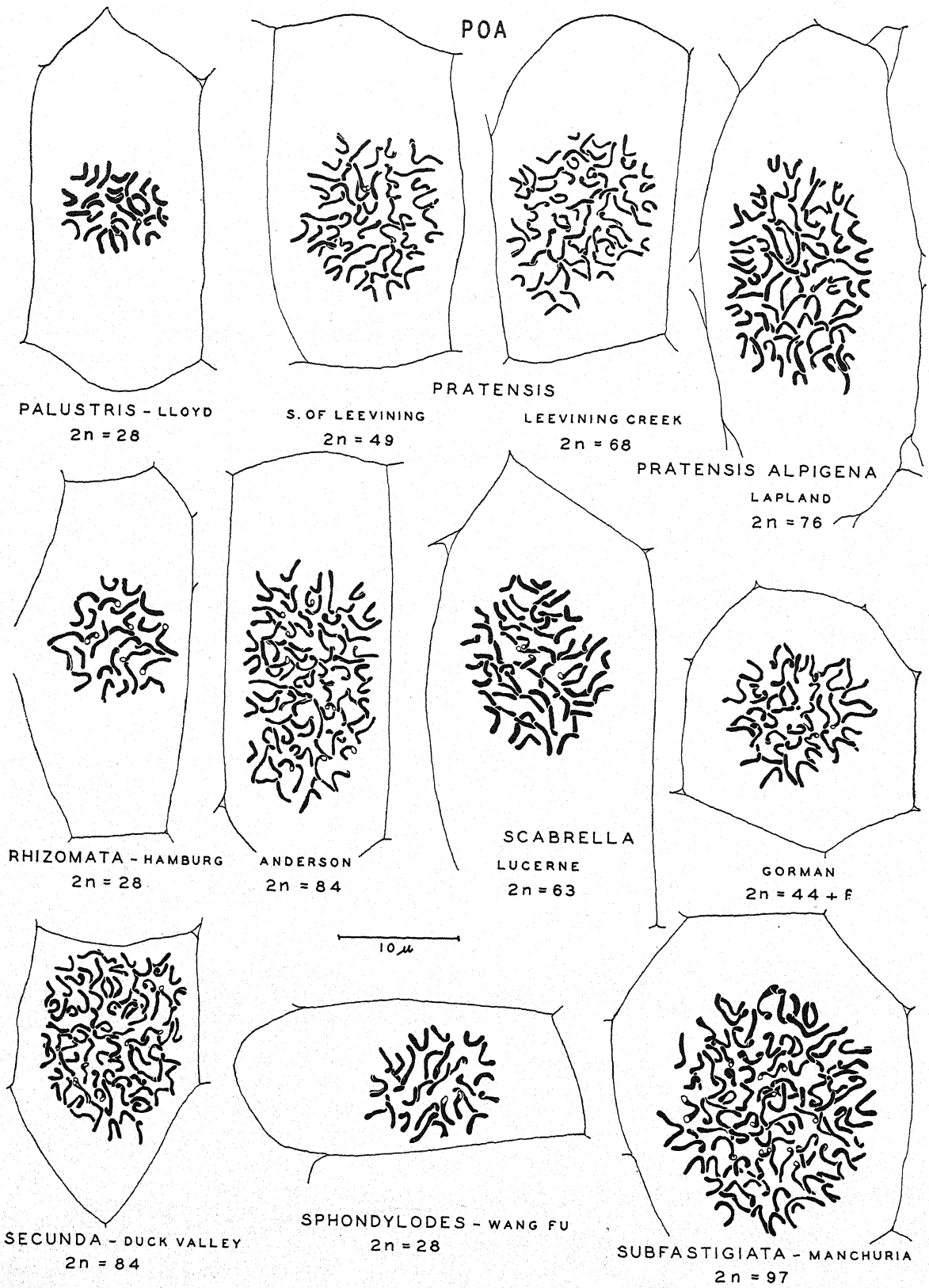


Fig. 3. Chromosomes of species of *Poa*.

robust and glaucous, has wider leaves and blooms later. It, too, usually has $2n = 84$ chromosomes (fig. 1), but the forms from higher altitudes tend to have a greater and more variable number, variations between 90 and 106 having been noted in one population. Like *gracillima,* with which this high altitude form is cohabitant, it differs from the low altitude forms by being summer-active.

Poa epilis and *P. Cusickii,* referred to the section *Epiles,* are bunchgrasses from the higher mountains. The former is a small range grass found in meadows and on rocky slopes around tree line. A plant from Oregon was 12-ploid, with approximately $2n = 84$ chromosomes (fig. 1), but Armstrong (1937) listed $2n = 56$ for two additional plants. *Poa Cusickii* is a larger plant of dry or rocky slopes. A form from Sierra Valley is hexaploid, with $2n = 42$ (fig. 1). Stebbins and Love (1941) reported $2n = 28$ for this species.

Poa palustris and *interior* are perennial grasses without rhizomes that belong to the section *Palustres.* The first is circumpolar in distribution. In the northwestern states and Canada it grows in meadows at low and medium altitudes. It is characterized by its large open panicles which top the tall, loosely tufted culms. The two races listed are from different continents and both have $2n = 28$ chromosomes. This number is the same as that obtained by Armstrong (1937) for two plants. Stählin (1929) and Avdulov (1931), however, determined hexaploids.

Poa interior extends across Canada and grows inland in the United States as far south as Arizona and New Mexico. Its culms are erect from the base and densely tufted. Two races, both from dry, gravelly slopes in Wyoming, were tetraploid (fig. 2) like most forms of *palustris.*

Poa sphondylodes, an Asiatic relative of these species, has a more congested inflorescence and very pubescent lemmas. Two strains, both from Suiyan Province, China, were tetraploid, with $2n = 28$ chromosomes (fig. 3).

Poa bulbosa is a European introduction that is erratically distributed over the United States. In Europe the species is found in dry woods, slopes and fields, and in this country it behaves as a ruderal in similar habitats. Also, in this country it is predominantly viviparous—the only such *Poa* here. An upland strain from Dickinson, Montana, is hexaploid, $2n = 42$ (fig. 1), and one occurring as a weed in the garden of the Soil Conservation Service at Pullman is approximately tetraploid. Armstrong (1937) recorded a tetraploid, $2n = 28$, and this number, as well as $2n = \pm 45$, was found by Åkerberg (1942).

A species from Armenia was received under the name of *Poa longifolia,* which is a native of the Caucasus region. The strain grown is a robust bunchgrass with wide, flat leaves, compressed, keeled sheaths, short ligule, and a large, open, pendulous panicle, with many florets per spikelet and smooth lemmas. *Poa longifolia* was described as being rhizomatous, with terete sheaths, narrowly linear, convolute leaves, and contracted inflorescences. The strain grown reacts like a montane species, for at Stanford it grows to a height of one meter, but at the Mather transplant station, at 4600 feet altitude in the Sierra Nevada, it increases to 160 cm., with leaves up to 80 cm. long and 5 mm. wide. The individual investigated had $2n = 43$ chromosomes (fig. 2). In smear preparations of meiosis in pollen mother cells 21 pairs and 1 single chromosome were clearly observed, suggesting that the species is sexual.

Poa pratensis, the Kentucky bluegrass, is the type species of the genus and the most important member of the rhizomatous section *Pratenses.* Of worldwide distribution, its success as a species is due to the fact that its forms can tolerate a great range of environmental conditions. The chromosome numbers for the species as it occurs in the wild have been reported by numerous investigators in various regions (Åkerberg, Armstrong, Avdulov, W. L. Brown, Flovik, Müntzing, Radeloff, Rancken, and Stählin). Scarcely any other species is known to show such variation in chromosome number, for the forms range from $2n = 28$ to ± 124, with almost all numbers from 42 to 87 represented. It is remarkable that with such an unusual cytological variation all of the forms are always recognizable as *pratensis.* The asexual mode of reproduction through apomixis is dominant in at least the higher chromosome types (Åkerberg, 1942). The chromosome numbers recorded for most of the races examined in the present survey were dysploid (table 1), with a range from $2n = 49$ to 84, the numbers being fairly evenly scattered, but with a distinct mode around $2n = 68$ and with indications of others around $2n = 49$–50 and 56. The chromosome number varies even within the local population, although most of its individuals have approximately the same number. Two types are illustrated in fig. 3.

An interesting maritime ecotype of *Poa pratensis,* from wind-swept bluffs along the Oregon coast, was picked up by Prof. W. E. Lawrence of Oregon State College. These plants were taken for the little known *P. Kelloggii* Vasey at first, for they had slightly longer and more rounded ligules and less pubescent lemmas than other forms of *pratensis.* It is now evident, however, that *Kelloggii* is a very different plant, largely confined to the habitats of the coast redwood in California. The maritime ecotype of *pratensis* is a low, dark green, wide-leaved plant that is winter-active in contrast with other forms of the species, which are winter-dormant. Its chromosome numbers are a little higher than those recorded for other forms of *pratensis* from western North America, namely, $2n = 81$ and 84 (fig. 2, upper right-hand corner).

Poa pratensis alpigena is a subarctic subspecies. Specimens collected by Dr. Jens Clausen in Swedish Lapland, north of the Arctic Circle, have grown well in California for years under very different climatic and soil conditions. The chromosome num-

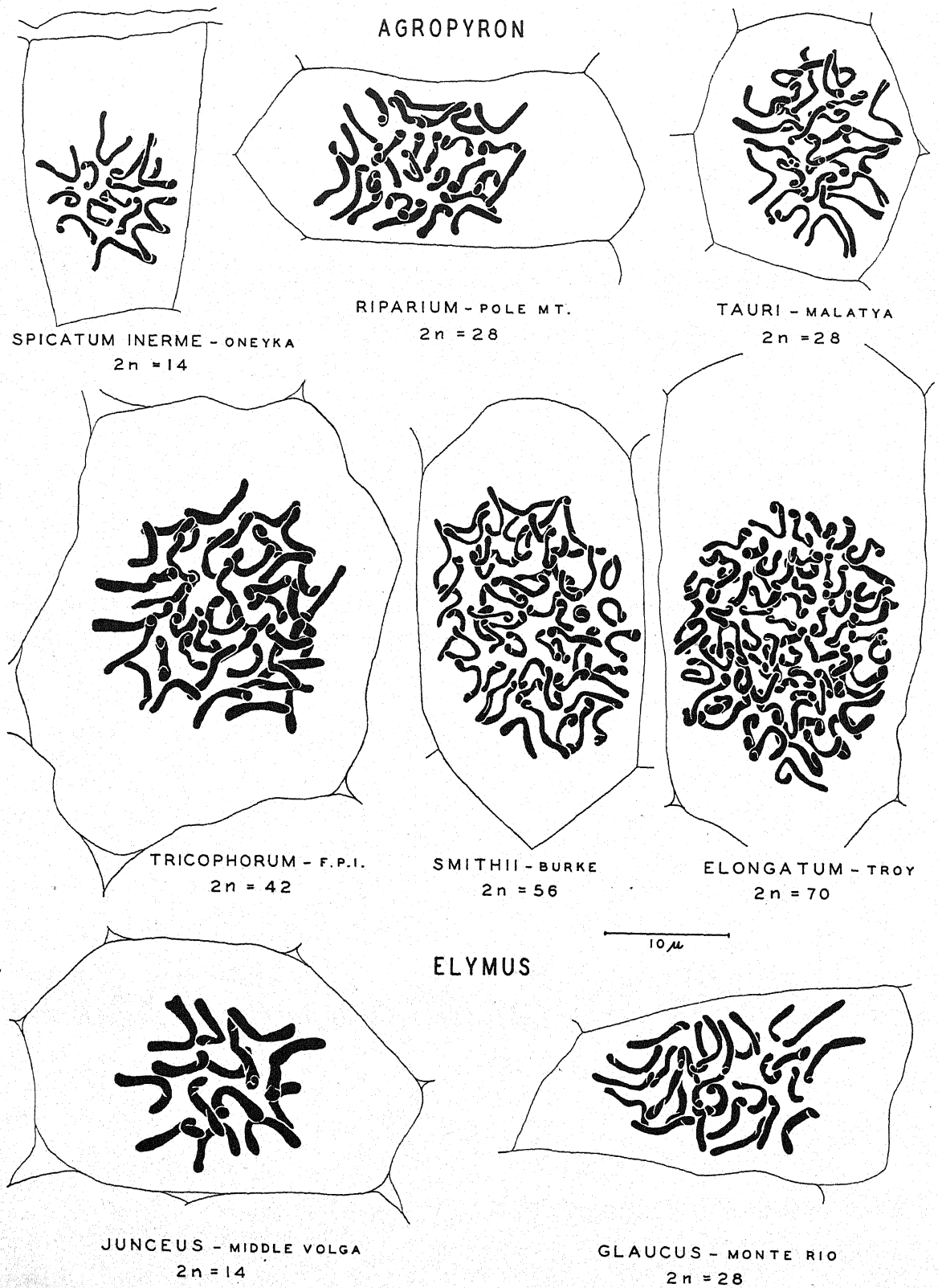


Fig. 4. Chromosomes of species of *Agropyron* and *Elymus*.

bers for 4 plants were determined approximately, the plate with $2n = 76$ being the clearest (fig. 3). Flovik (1938) has determined two strains with $2n = 51 + 5f$ and $42 + 4f$, and Åkerberg (1942) has counted two plants with 82 and 84 chromosomes.

Poa arida is the Great Plains counterpart of *P. pratensis* and the most valuable member of the genus in its region. It has glaucescent, rigid basal leaves, an inflorescence with shorter laterals than *pratensis,* and unwebbed lemmas. In the few samples obtained the nonaploid number of chromosomes predominates ($2n = 63$, fig. 1). Higher numbers were found, as in two dwarf plants with $2n = ca.$ 90 and 103 from a population in which the normal plant had $2n = 63$.

Poa glaucifolia inhabits most of the territory of *arida,* but occurs under moister conditions. The chromosome numbers $2n = 50$ and 56 in the two races investigated suggest that further counts will be needed to establish the pattern of variation in this species (fig. 2).

Poa fibrata is a newly described rhizomatous species that is thought to be of hybrid origin. Its appearance and distribution strongly suggest that a form of *P. pratensis* is one progenitor and one of *ampla* or *nevadensis* the other. The present material came from a meadow in Squaw Valley, western Lassen County, California, and is nonaploid ($2n = 63$, fig. 1) like either supposed bunchgrass ancestor.

Poa compressa, the Canada bluegrass, is an important pasture species grown frequently on poor soils in the Northeastern States and Canada. It has been introduced from Europe, where it is a species of dry hills and fields, and is now widely distributed in the United States. All three of the American races studied were hexaploid. This confirms Avdulov's (1931) count of $2n = 42$. Armstrong (1937) found $2n = 42$ and 56, and Åkerberg (1942) obtained three polyploid numbers, $2n = 35$, 42, and 49. Radeloff (1930) reports a diploid, with $2n = 14$. Our plant from Chorsum, Asia Minor, with $2n = 50$, is a fairly vigorous type originally from a dry clay hillside. The leaves are short and glaucous with anthocyanous tips, and the culms are not so characteristically flattened as in other members of this species.

Poa nervosa is chiefly a montane to alpine species with considerable range in the western United States and Canada but with relatively little variation. The plants in this investigation came from medium to high altitudes in the central Sierra Nevada, and all those examined have a $2n$ number of 62–64, excepting that from Ravine Creek, an alpine form that becomes viviparous when grown in the Carnegie Institution gardens at Stanford, which has $2n = 70$. All these plants were female. Stebbins (unpublished) has found $2n = 56$ in two plants in his collections.

A group of dioecious, prominently rhizomatous species includes *Poa arachnifera, Douglasii, macrantha,* and *confinis*. *Poa arachnifera,* native from Kansas and Arkansas to Texas, has now been introduced eastward through much of the South. It is an important range grass in that area on account of its winter activity. In it the two sexes differ in appearance, for the pistillate plants have conspicuously woolly lemmas and the staminate plants do not. Although Fargo and Woodward, Oklahoma, are but a few miles apart, two plants from the former are octoploid, with $2n = 56$ (fig. 1), and one from the latter is nonaploid ($2n = ca.$ 63). Brown (1939) determined material from Texas as having $2n = 42$.

Poa Douglasii ($2n = 28$), *macrantha* ($2n = 28$), and *confinis* ($2n = 42$) are all found on maritime dunes along the Pacific coast. The first two of these have long aerial runners unlike any other North American *Poa,* and all three species are valuable sandbinders. The chromosomes are illustrated in figures 1 and 2, and the count for *P. macrantha* agrees with that of Armstrong (1937).

Poa rhizomata is a rarely collected grass of limited distribution in the Siskiyou Mountains area of northern California and southern Oregon. Seedlings grown from a collection made in Siskiyou County, California, are not very active in the Stanford environment. A plate illustrating the $2n = 28$ chromosome number is found in figure 3.

Poa subfastigiata is a Manchurian species with long rhizomes like *pratensis* and with stiffly erect, thick and wide leaves. Like *pratensis,* it did not flower during its first year in the garden. Its high chromosome number of 91 to 97 was difficult to determine exactly, but its chromosomes are longer and larger than the average (fig. 3).

AGROPYRON AND ELYMUS.—These two genera also follow a 7-series of chromosome numbers, with diploid, tetraploid, hexaploid and octoploid representatives in both, in addition to a decaploid species in *Agropyron*. Presumably, these all reproduce sexually, and the cytological pattern differs from that in *Poa* in being strictly polyploid and very regular.

Sixteen species of *Agropyron* were investigated and are listed in table 2; six of these are illustrated in figure 4 as representatives of the polyploid series. A number of the species have been counted previously; these are indicated in the text.

Several of the species are composed of race complexes that differ in their number of chromosomes. One of these is *Agropyron spicatum,* which consists of diploid (fig. 4) and tetraploid races. It is distributed throughout much of the arid regions of western North America, so that the 19 races counted in this investigation represent but an incomplete sampling. It appears, however, that the tetraploid form has a relatively limited distribution in eastern Washington and adjacent northwestern Idaho, whereas the diploid populations occur from points as remote as northeastern California and Washington, north-central Nevada, and eastern Idaho. Robertson and Weaver (1942) reported a tetraploid form of *spicatum* from northeastern Nevada, but that appears to have been a local colony of limited distribution and possibly of a different origin. Like

the Nevada tetraploid, the five tetraploid races of the present investigations were markedly more vigorous than the diploids, although both types vary considerably in vigor. The tetraploids produce more leafy innovations, more and taller culms, and they tend to remain green longer than the diploids, which should make them more desirable as forage grasses. No other morphological differences were found to distinguish these chromosomal races. Peto (1930) determined the chromosome numbers of five plants from Alberta and found them to be diploid. This is one of the few Agropyrons that is predominantly diploid.

Agropyron inerme is here considered to be only an awnless variant of *spicatum,* for seven of the nineteen populations contained both *spicatum* and *inerme* plants as shown in table 3. It is to be noted that the *inerme* character occurred with about the same frequency in both diploid and tetraploid races of *spicatum.* From this tabulation it is evident that no taxonomic or cytologic significance can be attached to the awnless form in *spicatum,* although it has been widely regarded as a distinct species.

Agropyron Pringlei is a relative of *spicatum,* but with decumbent habit and shorter spikes; it comes from higher altitudes and rocky habitats in the Sierra Nevada. The strain investigated was collected near Glen Alpine in the Lake Tahoe region at about 8000 feet by G. L. Stebbins, Jr., who has confirmed our chromosome count (unpublished). During the first summer a culture of thirty plants developed well at Stanford, flowering in July and going dormant in the late fall, but died out during the mild winter.

Agropyron Smithii is another critical complex with two chromosomal races, in this case composed of tetraploids and octoploids, with respectively 28 and 56 chromosomes (fig. 4). This species, distributed across the Great Basin and prairie region, is very strongly rhizomatous. Forms from Washington to South Dakota, Kansas, and Texas were found to be octoploid, while the lone tetraploid came from southeastern Oregon on the periphery of the distribution, but the sampling is far too open to permit conclusions from a species of such a wide distribution. Peto (1930) found two tetraploids and twelve octoploids among fourteen plants from Alberta. It was impossible to discover any morphological differences between the two chromosomal races, but this species is the most variable of all Agropyrons. In each culture of thirty plants in these experiments there were scarcely two individuals alike, and the tetraploid race was as variable and as strong as the octoploids.

Agropyron riparium (fig. 4), *Elmeri,* and *dasystachyum* are all close morphological relatives of *Smithii,* but they are tetraploid, with $2n = 28$ chromosomes, as is *Griffithsii,* another close relative counted by Peto (1930).

A third complex consists of species that are native to the countries around the Mediterranean and in the Near East. In the present investigations this group is represented by *Agropyron tauri* ($2n = 28$, fig. 4), *intermedium* ($2n = 42$), *trichophorum* ($2n = 42$, fig. 4), and a decaploid form of *elongatum* ($2n = 70$, fig. 4).

Agropyron tauri is a little known species from the Taurus Mountains of Asia Minor. The strain investigated was collected 60 km. north of Malatya, a little north of the eastern spur of the Taurus. It was received under the name of *A. intermedium,* but differs from that species by its number of chromosomes, by being non-rhizomatous and much more slender, and by readily shattering the seed. All the other members of the group are coarse, robust species that at Stanford grow exceedingly well and remain green all summer even without irrigation.

The two races of *A. elongatum,* one from the Maikop region in the northern foothills of the Caucasus, and the other from the ruins of Troy at the entrance to the Dardanelles, consisted of extremely robust plants that developed some 150 to 200 reed-like culms 170–240 cm. tall. Peto (1930), Wakar (1934, 1936) and Simonet (1935a and b) all investigated Russian forms of this species and found them decaploid, but in addition Simonet found that the form from the maritime marshes of southern France is diploid ($2n = 14$). Host's type of the species came presumably from the Austrian Adriatic coast. The present investigation brings the decaploid as far west as the Dardanelles. Now it will be necessary to determine whether genuine *elongatum* is diploid or decaploid.

It is very unusual to have a diploid and a decaploid form within one species without intervening chromosome numbers, and Simonet found only slight morphological differences between the two. The intervening numbers, however, are known from closely related species of the same general region, *A. tauri* adding a missing number to the polyploid series. Three of these species are caespitose and non-rhizomatous, namely, diploid *elongatum* ($2n = 14$) from Mediterranean marshes, *tauri* ($2n = 28$) from the interior high plateau and mountains of Asia Minor, and the decaploid *elongatum* ($2n = 70$) from lower altitudes near the coast. The others are rhizomatous, although the rhizomes are short in *intermedium* and *trichophorum.* These are *A. littorale* (Host) Dum. ($2n = 42$, Simonet, 1935a) from maritime dunes, *intermedium, trichophorum* (both counted also by Araratian, 1938) and *glaucum* R. & S., three very closely related species all with $2n = 42$, and *campestre* Godr. & Gren. ($2n = 56$, Simonet, 1935a) from fields of France and the Hibernian Peninsula. An additional interest attaches to this group, because several species of wheat will cross with *glaucum* and the decaploid form of *elongatum* (Wakar, 1934, 1936).

Agropyron cristatum, crested wheatgrass, is a fourth species composed of two chromosomally different race complexes, in this case of diploids and tetraploids, with $2n = 14$ and 28. This species has a natural distribution from southeastern Europe to Altai and Baikalia in Siberia, where it occurs in

rather dry, sandy localities, and it has been introduced as a hay and pasture grass in the northern Great Plains and the intermountain region of the United States. Peto (1930) found four diploids and five tetraploids among strains received from sources in the U.S.S.R. and North America; Avdulov (1931) reported a tetraploid but did not give its origin; and Simonet (1935a) counted a diploid. Three of the strains of the present investigation were from Asia Minor and the Middle Volga, and two were American cultivated forms. The total cytological sampling to date has been too spotty and the native habitats too little known to furnish reliable information on the distribution of the two races of this species. Neither Peto nor we detected obvious morphological differences between the diploids and the tetraploids.

Agropyron sibiricum and *Michnoi* are close relatives of *cristatum,* but both are tetraploid. The two strains of *sibiricum* came from the steppe region north of the Aral Sea in Kasakstan. Peto (1930) likewise found that 2 strains of this species and eight plants of the closely related *A. desertorum* were tetraploid.

Agropyron caninum, subsecundum, and *pauciflorum* are tetraploid bunchgrasses belonging to a circumboreal complex of closely related species that are constant in chromosome number, for all have $2n = 28$. *A. caninum* is a Eurasiatic species of considerable variation, occupying habitats from low to high altitudes and latitudes. The present determination verifies those of Peto (1930), Avdulov (1931), and Simonet (1935a).

Agropyron subsecundum and *pauciflorum* are the North American counterparts of *caninum.* They differ from each other mainly in awn-length, *subsecundum* having a long awn like *caninum,* and *pauciflorum* having awnless or merely awn-tipped glumes and lemmas. This difference parallels that between *spicatum* and *inerme.* The long-awned form is rare in California, but at a mid-altitude meadow in the Sierra Nevada at Mather both forms are together. Seven plants from this habitat were counted—four awnless or awn-tipped and three awned. In table 2 all are listed under *pauciflorum.* Offspring resulting from open pollination in the Mather meadow were grown in the Stanford garden from six of the seven plants. The awnless plants produced only awnless offspring, and two of the long-awned plants produced only long-awned offspring, but one awned plant segregated some awnless offspring in addition to others with awns of varying length. This segregation was not correlated with sterility. Peto (1930), listing *subsecundum* as *Richardsonii* Schrad. and *pauciflorum* as *tenerum* Vasey, found 35 plants of the former and 36 plants of the latter from various parts of Alberta were tetraploid, with $2n = 28$ chromosomes. He also reported that an artificial hybrid between the two was sterile, but that it had 14 pairs of chromosomes in meiosis. Avdulov (1931) and Nielsen and Humphrey (1937) reported 28 chromosomes for plants of *tenerum* without mention of locality, and Stebbins and Love (1941) counted $2n = 28$ in four strains of *pauciflorum* from California. Three of the present strains were received from Stebbins. The taxonomic status of *subsecundum* and *pauciflorum* cannot be settled before their distribution, variability and possible interfertility in various parts of their distribution are better known.

Members of the genus *Elymus* follow the cytological pattern of *Agropyron,* but the tetraploids more definitely predominate. Out of 58 races of *Elymus* counted, 57 were tetraploid ($2n = 28$) and one was diploid ($2n = 14$). The various races are listed in table 4. Avdulov (1931) reported higher numbers in two species, namely, $2n = 42$ in *E. dahuricus* Turcz. and $2n = 56$ in *E. arenarius* L.

Elymus glaucus is a highly variable, tall, coarse bunchgrass occupying a variety of habitats in North America from the Great Lakes to the Pacific. Within a single population notable variations in color and type of foliage and resistance to disease occur. Strains ranging from the immediate coast to 10,000 feet altitude in the Sierra Nevada and from southern California to Washington and Idaho were included in the present survey. The chromosomes of a pubescent strain referable to var. *Jepsoni* Davy, from Monte Rio along the Russian River, are shown in figure 4. Stebbins and Love (1941) also found 28 chromosomes in fourteen races of this species from eight California counties. Eleven of the 35 races reported here were received from Stebbins.

Elymus glaucus appears to be fairly closely related to the *Agropyron subsecundum-pauciflorum* complex, which occurs in the same territory. Principal differences between the two are that the *Elymus* has two or three spikelets per node and a somewhat longer awn; also it flowers a little earlier. At Mather, the *Elymus* grows on slopes adjacent to the meadow where *Agropyron pauciflorum* and *subsecundum* occur, and rarely an *Elymus* plant is found right with the *Agropyron.* Sterile individuals with predominantly single spikelets at the nodes but with longer awns than the Mather form of *subsecundum* also occur in the meadow. Stebbins (unpublished) interprets these as belonging to the hybrid *A. pauciflorum* × *E. glaucus.* One such plant was brought to Stanford where it grows well but remains sterile. It has 28 chromosomes in its root-tips, although some cells have 29, possibly on account of fragmentation. Some cytological confirmation of the hybrid theory was obtained from this plant, for in smears of the pollen mother cells both multivalents and single chromosomes were observed in addition to normal pairs.

Elymus virescens is a coastal, essentially awnless relative of *glaucus,* and may even prove to be a maritime ecotype of that species. The strain grown had prostrate culms in keeping with its maritime origin. Stebbins (unpublished) has also found $2n = 28$ in this species from Trinidad, Humboldt County, California.

Elymus canadensis is also related to *glaucus,* but

has pubescent lemmas, firmer glumes, nodding spikes, and divergent awns. It occurs in many and varied habitats across the North American continent, and in the present study no chromosomal variation was discovered in thirteen races within the area outlined by localities as remote as Oregon, Washington, Montana, Kansas, and New Mexico. They confirm the findings of Avdulov (1931) and Nielsen and Humphrey (1937), who also found this species tetraploid. The latter authors also recorded a hexaploid, with $2n = 42$, but the unusually small chromosomes they illustrate could belong to *Stellaria media,* a common 42-chromosome greenhouse weed.

Elymus triticoides is a rhizomatous species of moist soil inhabiting all of the western states. In the present study races were grown from climates as different as those of the outer Coast Range, mid-altitude in the Sierra Nevada, the Great Basin of California, eastern Oregon and Washington, and Idaho, and all were found to be tetraploid, $2n = 28$. Stebbins and Love (1941), however, reported six tetraploid and two hexaploid forms, and Gould (1945) an additional eleven tetraploids and ten hexaploids, all from cismontane California. According to Gould, the very large hexaploid plants are mainly coastal.

The form here listed as *Elymus cinereus* came from a dry pumice slope at the east base of the Sierra Nevada, where it grew as a bunchgrass among the sagebrush. More than sixty seedlings have been grown, all from one small but variable colony, the seed of which was fairly highly sterile. The seedlings were variable in vigor, presence or absence of pubescence and rhizomes, etc., and the four plants cytologically studied represented different combinations of these characters, but all were tetraploid ($2n = 28$). The only other awnless *Elymus* in this general area is *triticoides,* which grows in moister habitats but at times in proximity to this more slender dry slope form. Possibly the strain investigated is a hybrid swarm between *cinereus* and *triticoides.* Gould (1945) found 28- and 56-chromosome forms in the Great Basin material of *cinereus* from Oregon, Washington, and Utah and reported the same variation in chromosome number in the closely related but much more robust *E. condensatus* Presl from the California Coast Range. Gould's paper serves to emphasize the fact that much critical investigation is still needed before we can begin to understand the *Elymus triticoides-condensatus-cinereus* complex.

Elymus junceus (fig. 4) from Russia is the only diploid member of the genus thus far reported. It grows at Stanford as a tall, divaricate-erect grass with blue-green herbage, the leaves being rather soft and narrow as compared with other species of *Elymus.* The rosette is dense as in *Poa,* and active all summer after the culms have dried, which is in contrast with the behavior of *E. glaucus,* the most common West American species. This characteristic enhances its value as a range grass and as a stock for improving the western species by breeding.

Discussion.—Of all the Poa species investigated, *P. pratensis* is no doubt the most highly evolved. Races representative for the most part of medium altitudes in California present a picture similar to that found in this species by Müntzing (1933, 1940), Flovik (1938), Åkerberg (1936, 1942, 1943) and others. Extreme polymorphism is accompanied by a long series of chromosomal variations.

The occurrence of different chromosome races in apomictic species is not uncommon, but the very extensive dysploid series found in *Poa pratensis* is very unusual. It is possible that autoploidy and alloploidy both have played a part in the building up of this species from one or several primary types. Åkerberg (1942) considers $2n = 49$ to be a primary type from which all higher numbers could have been derived. It is not possible to determine exactly to what extent this or any other primary type is auto- or alloploid because of the advanced stage of the evolutionary process.

The other species of *Poa* are less variable than *pratensis.* Those like *P. ampla, nervosa,* and *nevadensis* seem to be rather consistent in maintaining the chromosome number at a certain polyploid level with only slight deviations from the cardinal number. Apomictic reproduction keeps the number constant, and occasional accompanying sexual reproduction produces gametes that give rise to the polyploid variations sometimes found, but these are usually weak and are often unable to survive even in space plantings. The *Poa ampla* from Albion, Washington, for example, usually has $2n = 63$, but plants with other polyploid numbers have been found including the one with $2n = ca.$ 100 listed in table 1. Occasionally such variants are strong and establish themselves as a population, as, for example, the 70-chromosome race of *ampla* from Heise Hot Springs, Idaho, and one of *nevadensis* from Winchester, Idaho, also with 70 chromosomes, 7 more than the normal number.

The forms of *Poa scabrella* appear to vary around two polyploid levels, 9- and 12-ploid, with occasional extremes such as the hexaploid from Gorman and the 15-ploid from Beegum. Within a single local population, adequate sampling yields some plants which are extreme variants and some which deviate only slightly from the average of the sample, *e.g.,* the population from northeast of Mather.

There is apparently a basic combination of genomes which is necessary to produce the normal type of a *Poa* species. In most species with higher chromosome numbers partial or complete duplications of the basic genomes do not modify the specific characteristics appreciably. A number considerably lower than the basic number, or one extremely higher, will produce weak or slow-growing aberrant plants.

Almost no evidence could be found in the material presented in this paper for the opinion that there are geographical or ecological differences

between the chromosomal races. However, the races of *Poa Canbyi* from the north have a consistently lower chromosome number than those from the Sierra Nevada at tree line.

The multiplicity of forms and the intergradation of species found in *Poa* present a state of taxonomic confusion which defies any attempt at solution on a purely morphological basis. In such a group of plants, it is only through an analysis of the species complexes, using a combination of cytogenetic, ecologic and morphologic data, that a natural basis for classification can be approached.

SUMMARY

The chromosome numbers of 26 species of *Poa*, represented by a total of 145 races, are reported. Of these there are 33 races of *Poa pratensis*, 24 of *ampla*, 18 of *scabrella*, 12 each of *Canbyi* and *nevadensis*, and 6 or less of each of the other species. From the counts given and those reported by others, intraspecific chromosomal variation was established in all but 7 of the species. *Poa* species follow a 7-series in their chromosome numbers, but since many of them reproduce by apomixis, which tends to perpetuate chromosomal irregularities, aneuploid deviations from the polyploid series occur frequently.

Agropyron and *Elymus*, which reproduce sexually, also follow a 7-series, but aneuploid counts in them are very rare. The chromosome numbers of 16 species (69 races) of *Agropyron* are reported. These agree in general with those published previously and form a complete polyploid series from diploid to decaploid. Four of these species of *Agropyron* have two chromosomal races each, namely, *A. spicatum, Smithii, elongatum,* and *cristatum*. On the basis of the experimental data, the validity of *A. inerme* as a species apart from *A. cristatum* is questioned. Six species of *Elymus* were examined cytologically and all were tetraploid, with $2n = 28$, excepting *E. junceus*, which was diploid.

Previous chromosomal counts on these species of the three genera are mentioned.

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STUDIES OF DEVELOPMENT IN LONG SHOOTS AND SHORT SHOOTS OF GINKGO BILOBA L. II. PHYLLOTAXIS AND THE ORGANIZATION OF THE PRIMARY VASCULAR SYSTEM; PRIMARY PHLOEM AND PRIMARY XYLEM [1]

James E. Gunckel and Ralph H. Wetmore

THE FIRST part of this investigation has dealt with the origin and the development of cortex, pith and procambium in long and short shoots of *Ginkgo biloba* L. Leaf primordia were shown to arise in an orderly sequence on the shoot apex. They were preceded, and perhaps stimulated to initiation, by the continuous, acropetal development of the procambium into the zone of incidence of leaves. This acropetal development originates from older traces in the axis below. It is, therefore, necessary to consider the phyllotaxis of the shoot if one is to determine whether these origins are random or regular. Once the relationship of the vascular strands to shoot organization has been established, then the orderly sequence of primary xylem and primary phloem elements in the vascular strands can be followed effectively. The present study considers (1) the phyllotaxis and the progressive acropetal development of the primary vascular strands, and (2) the orderly development of the vascular elements in these strands. For the origin and development of the primary vascular tissues, the terminology and concepts of Esau (1943a) have been adopted.

The collecting of materials, the methods of preparation of materials for study, and the techniques for the reconstruction and for the determination of phyllotaxis in the stem tips from serial longitudinal and transverse sections have been considered in the first part of this series (Gunckel and Wetmore, 1946).

PHYLLOTAXIS.—In the first part of this investigation (Gunckel and Wetmore, 1946), it was pointed out that, as the plant grows, the apical meristems, by progressive development, add increments to the stem and branch apices which continue the growth patterns already established in the shoot below. The procambial strands of *Ginkgo* develop continuously and acropetally from definite procambial strands already present in the lower axis. It was indicated that, of the two procambial strands to each leaf, one strand is derived from the flank of a trace belonging to the next older leaf in the same "spiral", and the other from a vertical branch off an adjacent "spiral".

Because of the difficulty in recognizing the procambial strands destined for very young or presumptive leaves, evidence was provided for only the strands to leaf -1 (Gunckel and Wetmore, 1946). Here, the acropetal extension of these strands could be pictured by the pattern of active longitudinal divisions in the peripheral subsurface layers of the stem apex. However, the two procambial strands to any leaf became more easily recognizable when followed in serial transverse sections below the level of the stem apex. In this fashion, it has been possible to locate and establish the traces for leaves -1 to -5 and to demonstrate (fig. 10–12) their actual relations to the primary vascular skeleton (fig. 2). Thus, at 60 micra below the apex (fig. 11), the positions in the eustele which will be occupied by leaves -1 to -5 are clearly evident though the procambium itself is not recognizable at this magnification. However, at a level 60 micra lower (fig. 12), the seriate arrangement of procambial strands is quite apparent for the "double bundles" of leaves -1 and -2, is not so clear for those of leaves -3 and -4, and is not at all evident for leaf -5, though it becomes progressively so in successively lower sections.

Serial transverse sections of the stem apex permit one to follow the young leaves from tip to buttress. They also clearly demonstrate the orderly sequence of leaf appearance. In a transverse section taken just at the stem apex, the leaves or their traces may be numbered, beginning in the center, in the order of increasing age, the youngest primordium being arbitrarily given the number 1. Figures 1 and 10 illustrate the orderly, plastochronic succession of leaves on a terminal, long shoot apex.[2] The curviserial arrangement of the leaf members in any series, e.g., that represented by the traces in leaves 1, 6, 11, 16, etc. (fig. 1), indicates a general spiral course characteristic of that series at the level of the stem apex. Thus leaf 1 arose on the stem apex 5 plastochrones after leaf 6, leaf 6 five plastochrones after leaf 11, etc. If, in serial sections, the successive leaf traces of each "spiral" be followed basipetally to their respective "junctions" with vascular strands in the axis below, the relations of each trace to those of the same "spiral" and to traces of other "spirals" may be reconstructed. In figures 4–8, the vascular strands of leaves 1 and 6 (fig. 1), followed in serial transverse sections, will serve to show the essential pattern of vascular organization in *Ginkgo*.

Figure 4 shows the position of leaf 1 between leaf 4 and leaf 6. Here, at the level of the stem apex, no easily recognizable procambial strands for leaf 1 are yet evident, though there are clearly two strands for leaf 6. Just 60 micra below the stem apex (fig. 5, pc:1) the procambial cells of the traces to leaf 1 become evident, appearing slightly elongated, since at this level the traces are oriented almost parallel to the plane of section. Because of the oblique course, the double nature of the vascular system to leaf 1 is not very evident here. The vascular supply to leaf 6 appears double at this level,

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[2] It is to be noticed that the arrangement of leaves on the apex represented in figure 1 is the reversal of that in figure 10. From a consideration of some 200 stem apices, it appears that the dextrorse and sinistrorse patterns of leaf arrangement are about equally frequent.

as it is cut transversely. A transverse section cut 100 micra further down (fig. 6) shows that the procambial system for leaf 1 now appears nearer to the place which it will occupy in the cylinder, forming an arc flanked by the traces of leaves 9 and 6. At this time, the vascular system of leaf 6 occupies a slightly oblique position so that its double nature is less apparent. The traces of leaf 11 are nearly flanking those of leaf 6 on the anodic[3] side. At 220 micra below the stem apex (fig. 7), the traces for leaf 1 are shown as quite separate just above the gap region for the now approximate traces of leaf 14, one trace becoming confluent with the flanking trace of leaf 9 and one with a trace from leaf 6. The double nature of the vascular supply to leaf 6 is still obscure because of the oblique course but a V-shaped tongue of parenchymatous, vacuolated cells (*tp*) on the pith side gives evidence of its existence. One side of this double bundle is nearly confluent with a trace from leaf 11, the other, as just indicated, with one of leaf 1. Finally, in a section 540 micra below the stem apex (fig. 8), the two traces of leaf 14 have occupied the space in the primary vascular cylinder directly below leaf 1. The trace for leaf 19 is shown here near its "level of

[3] The direction along the phyllotactic "spiral" towards the successively younger leaves is designated *anodic* and the opposite direction, *kathodic*.

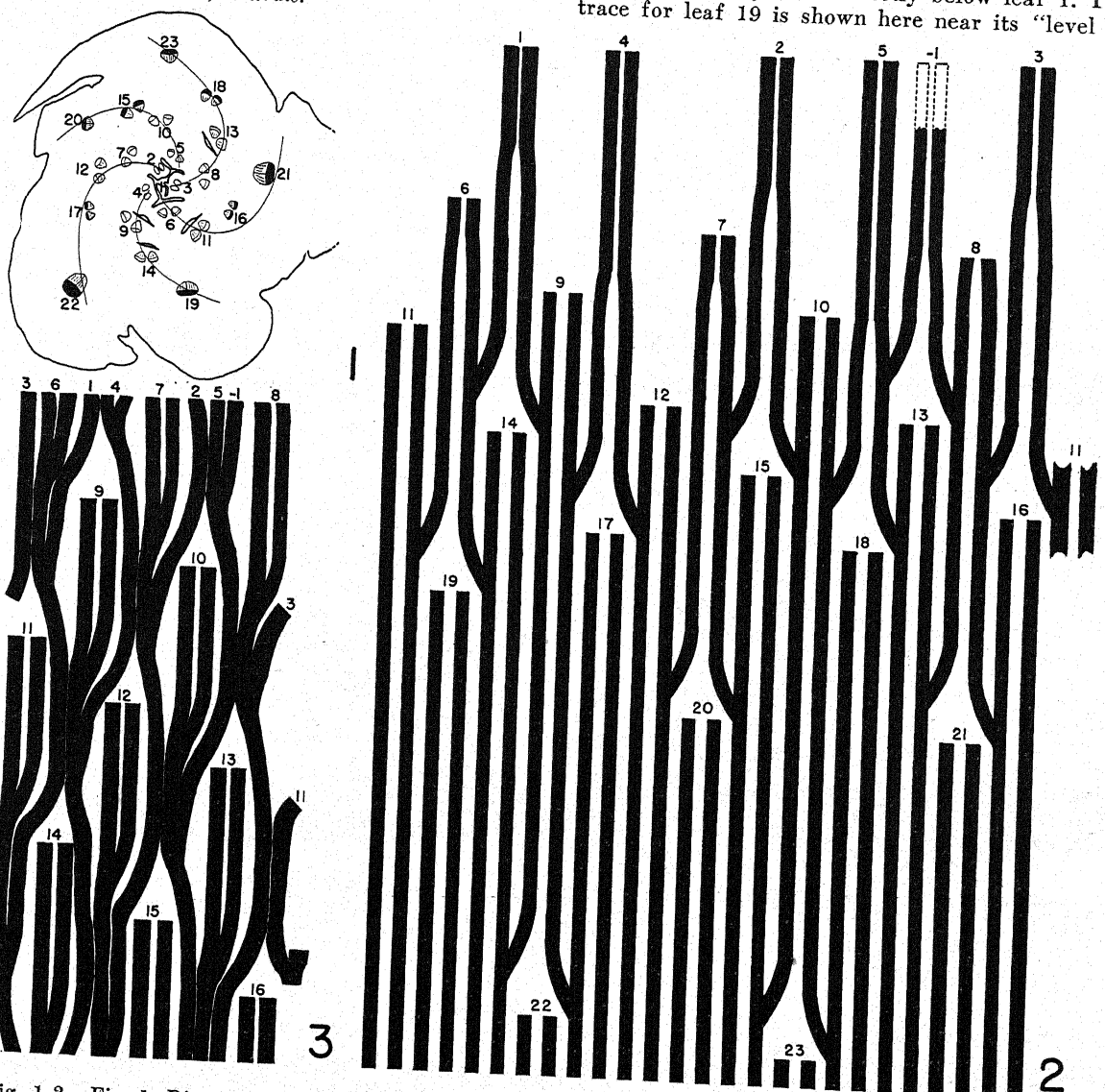


Fig. 1–3.—Fig. 1. Diagram of the transverse section from a lateral short shoot taken just at the stem apex. The phyllotactic "spiral" is $\frac{5}{13}$ or 5 + 8. Beginning with the youngest as leaf 1, the leaf traces are numbered in plastochronic succession. —Fig. 2. Diagram to show the vascular cylinder of a terminal short shoot of *Ginkgo* as though the stem was slit open lengthwise and spread on a plane surface. The diagram shows the level of leaf insertion and the level at which the two traces of each leaf made their respective "junctions" with vascular strands in the axis below.—Fig. 3. Diagram (after Geyler) to show suggested leaf trace origin for *Ginkgo*, by division of a single common stem bundle, in contrast to the condition portrayed in figure 2.

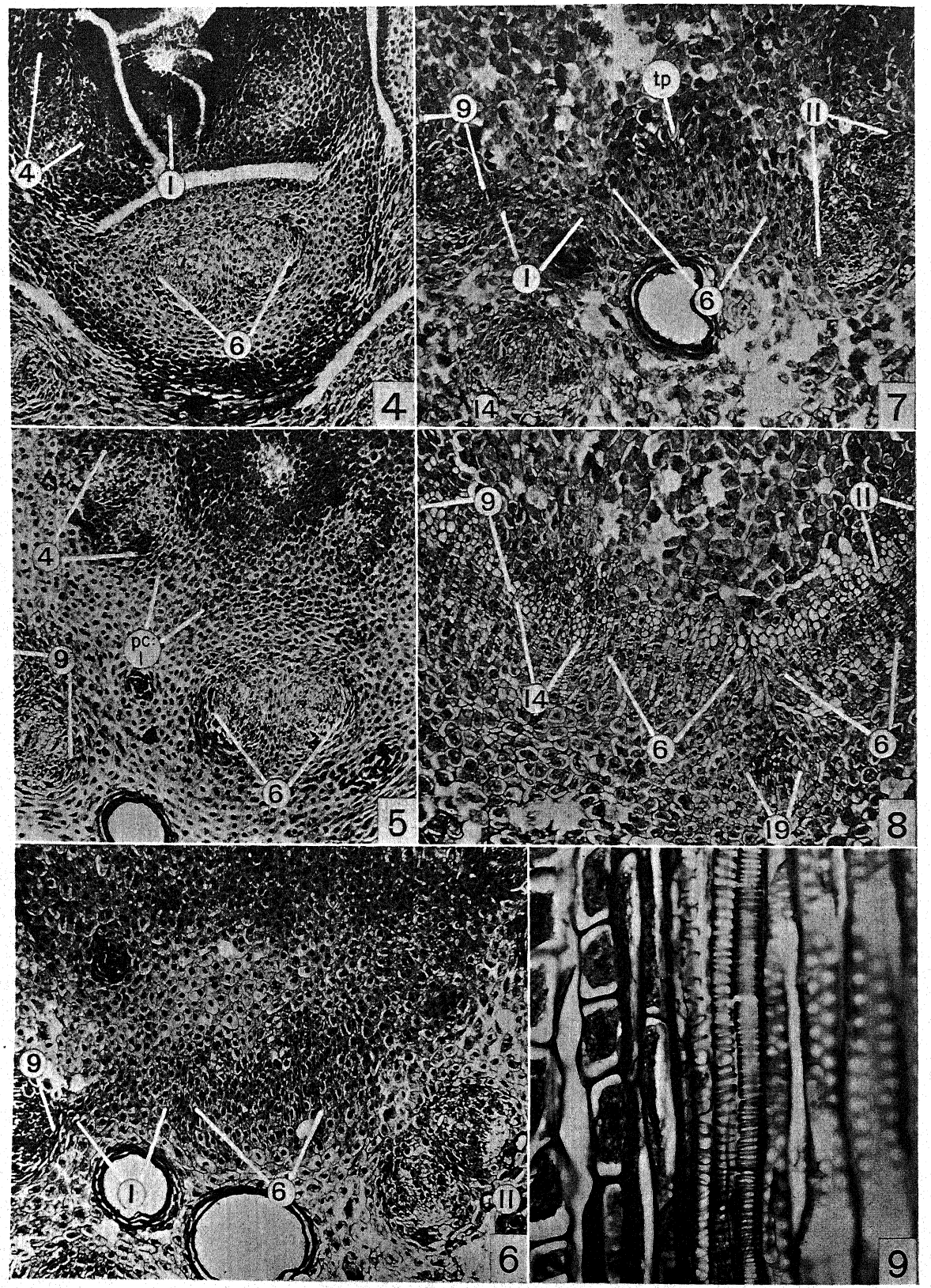


Fig. 4–9.—Fig. 4–8. Transverse sections of lateral short shoot to show the pattern of vascular connections for the

confluence" with the vascular cylinder. Just above the gap for this trace, one vascular strand of leaf 6 has become confluent with one of leaf 11 (anodic) and one—sympodial of traces from leaves 1 and 6 (fig. 2)—with a strand of leaf 14 (kathodic).

The pattern of development demonstrated in this apex was evident in all apices studied except for such variations as accompany reversals of phyllotactic sequence. In plastochronic succession, each leaf or its traces was followed basipetally in serial transverse section. In each case, with the apex as a level of reference, data were compiled to show: (1) the level of leaf insertion, a somewhat arbitrary level across any leaf base, proximal to which the two strands to that leaf can be considered as foliar strands[4]; (2) the levels at which the two traces of each leaf make their respective "junctions" with vascular strands in the axis below. The observations on a single tip are diagrammatically presented (fig. 2) as though the stem was slit open lengthwise and spread on a plane surface. For example, the anodic trace of leaf 1 is connected with a flanking trace of leaf 6 in its own "spiral", the kathodic, with the anodic trace of leaf 9 in the adjacent "spiral". Leaf 9 arises 13 plastochrones after leaf 22 and directly above it though in an adjacent "spiral". The two traces to leaf 9, therefore, originate on the flanks of the gap for leaf 22 and continue through 13 internodes before they diverge into the leaf. The kathodic trace of leaf 9 is related only to leaf 9 in the upper eight internodes of its course, that is, down to node 17, but is a sympodial (= compound) strand in the lower five internodes since here it is sympodial with the anodic trace to leaf 4. The anodic trace of leaf 9 is a sympodial strand with the kathodic trace of leaf 1 in the lower eight internodes of its course, that is, from node 14 to 22. It should be noted particularly that, of the two leaf traces of a leaf at any level, the trace provided by the adjoining "spiral" is more highly developed and branches off slightly lower in the axis than that derived from its own "spiral", e.g., the kathodic trace of leaf 5, than the trace provided by the next older member in the same "spiral", the anodic trace of leaf 5 (fig. 2, 13). One must emphasize the fact that the two traces in any one leaf are always morphologically distinct in their vascular connections.

[4] Following Esau's (1943b) practice, the entire course of each vertical strand to any one leaf, from its incidence at the level of the leaf directly below it to its divergence into its own leaf, is here referred to as a *leaf trace*.

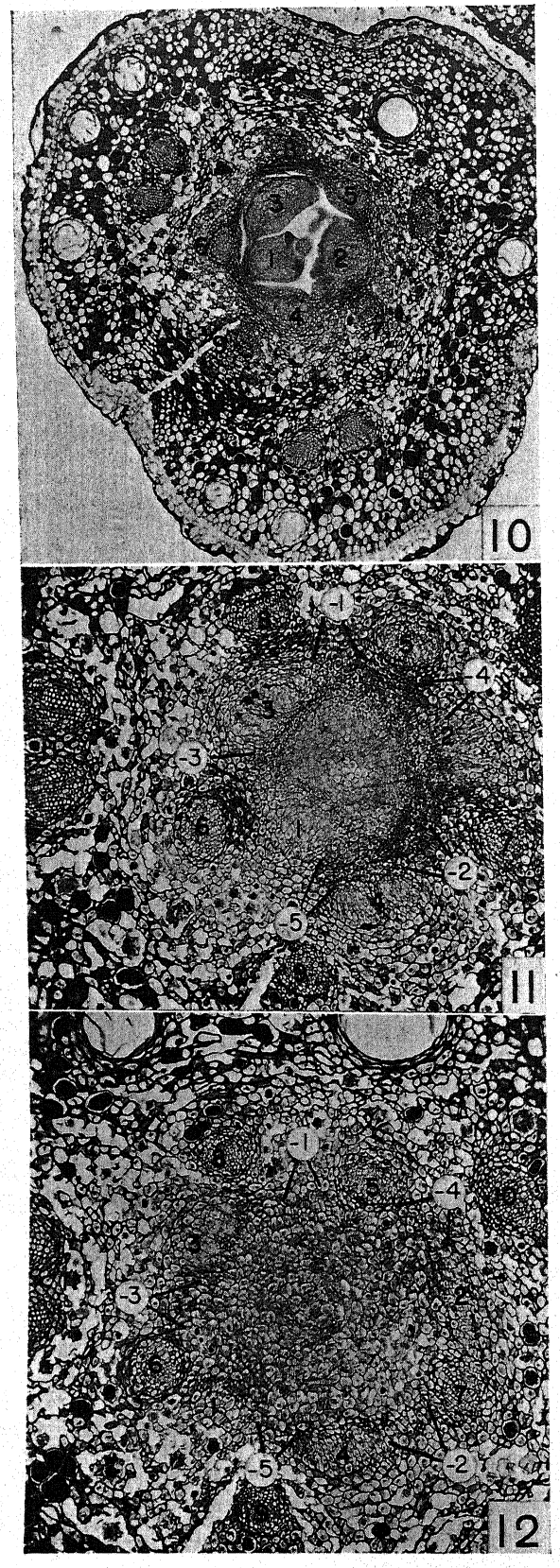
Casual inspection of transverse sections of *Ginkgo* apices might give the impression that some leaves have only one trace, particularly in seedling apices or in the younger leaves of mature apices. In such cases, the procambial strands may not yet be well established and therefore not clearly demarcated or the oblique path of the traces in their upper course toward the apex may obscure their double nature, but careful examination always shows a plate of parenchymatous cells, at least one cell wide, between the two traces to any leaf.

The sympodial leaf traces diagrammed in figure 2 are in contrast to those described for *Ginkgo* by Nägeli (1858), Geyler (1867) and Barthelmess (1935). Geyler maintains (fig. 3), in contradistinction to what has just been described in this investigation, that the leaf trace arose as a single branch from a common bundle in the same spiral series and then divided dichotomously into the characteristic two strands extending up into the leaf.

PRIMARY PHLOEM AND PRIMARY XYLEM.—As already indicated (Gunckel and Wetmore, 1946), a transverse section of a stem apex shows progressive stages of development in the successive, "spirally" arranged leaves. Thus leaves 1 through 4 give evidence on the origin, the expansion and the circumferential delimitation of the procambial strands (Gunckel and Wetmore, 1946). The sequence of events in the development of the primary phloem and xylem from their procambial precursors will be described for leaves 5 to 8. This sequence of events proved very difficult to establish in detail for the phloem region since changes are numerous, diverse, and apparently rapid. The following report is, therefore, submitted with reservations. Until some more specific method is available for recognizing mature sieve cells in the general phloem region, it will always be difficult to be sure of one's interpretation of elements in as heterogeneous an aggregation of cells as the young phloem region of *Ginkgo* proves to be.

The outer cells in the phloem region increase markedly in both length (fig. 17, 18) and diameter (fig. 13, 14) and become apparent in longisection as vertical rows of short cells. In both long and short shoots, a varying number from the outermost of these cells become early recognizable through their dark-staining contents, probably phenolic in nature. Such cells are more numerous in long than in short shoots; short shoots tend rather to produce

strands of leaves 1 and 6. All ×94.—Fig. 4. Section through apex of stem showing position of leaf 1 between leaf 4 and leaf 6. Leaf 1 has no recognizable procambial strands at this level; leaf 6 has two strands.—Fig. 5. Section cut 60 micra below the stem apex. Note procambial cells (*pc*:1) in traces of leaf 1 and leaf 6.—Fig. 6. Section cut 160 micra below the stem apex. The traces for leaf 1 now occur in the vascular cylinder and are flanked by those of leaves 9 and 6.—Fig. 7. Section taken at 220 micra below the apex. The traces for leaf 1 are noticeably farther apart just above the gap for leaf 14, one trace becoming confluent with the flanking trace of leaf 6 and one with a trace from leaf 9. A tongue of parenchyma (*tp*), continuous with the pith, occurs between the traces of leaf 6. One trace of leaf 6 is nearly confluent with a trace from leaf 11.—Fig. 8. Section cut 540 micra below the apex. The traces for leaf 14 have occupied a place in the cylinder directly below leaf 1. Above the gap for leaf 19 one trace of leaf 6 has become confluent with a trace of leaf 11 and one with a trace of leaf 14.—Fig. 9. Longisection of leaf buttress. From left to right the section shows elements of helically-thickened protoxylem, pitted metaxylem and circular-bordered pitted metaxylem. The early protoxylem elements show signs of having been stretched. ×300.



druses of calcium oxalate in these outermost cells. Unlike other elements in the phloem, these dark-staining cells do not become crushed, persisting at least throughout primary activity and effectively marking the outer limits of the vascular strands.

Other cells of the outermost phloem region, even some in the same vertical series as those just described, become fibrous as well as dark-staining in nature (fig. 18, *f*). Even near the apex, these fibers acquire taper-pointed ends and undergo further elongation until they may attain a length of a millimeter or more, suggesting sliding or intrusive growth. At about the time that metaxylem is being established, these cells with their dark-staining contents deposit very thick, lamellated secondary walls. Though quite common in long shoots, fibers were few in number in the lateral short shoots examined.

While certain of the outermost, large, procambial elements give rise to the dark-staining and the fibrous cells just described, others, centrad to the last, progressively undergo those developmental changes which ordinarily lead to sieve cell[5] formation (fig. 14, 15, *sc*). These cells show the characteristic peripheral disposition of the thinned-out protoplasts, the loss of nuclei, some thickening of the cell walls and the presence of sieve areas on the side walls, all well recognized characters of mature sieve cells (Esau, 1939). It should be mentioned that the sieve areas are less numerous and are stained with more difficulty than are those on the meta- or secondary phloem sieve cells (fig. 18, *sc, ssc*). The large diameters and thinner walls are in striking contrast to the protophloem elements reported for Angiosperms. It is also uncertain whether all of the potential sieve cells in this region eventually lose their nuclei. However, certainly some of them, irregularly placed among these enlarged cells, qualify as mature sieve cells under the definition of Esau (1939) (fig. 14, 15, 18, *sc*).

At about the time when the outer cells in the phloem region are enlarging, radial rows of xylem mother cells are produced in the middle of each strand; afterwards other rows form progressively on either flank (fig. 13, 14). Polarized periclinal divisions across the middle of the traces add cells on the phloem as well as on the xylem side in the ratio of about 1:2 respectively. The large mature sieve cells (fig. 14, *sc*) in the outer phloem region

[5] Following Cheadle and Whitford (1941), single functional units of phloem, derived from procambial or cambial fusiform initials, are termed *sieve cells;* vertical rows of functional phloem cells are designated *sieve tubes* and their component cells, *sieve tube elements.*

Fig. 10–12.—Fig. 10. Cross section of terminal long shoot cut at the level of the apex. The phyllotactic "spiral" is $5/13$ or 5 + 8 but a reversal of that shown in figure 1. The positions of leaves 5, 6 and 8 (fig. 13–15) are indicated. ×30.5.—Fig. 11. Section cut 60 micra below the stem apex. The positions of the leaf traces to existing leaf primordia and of the less evident leaf traces to presumptive leaves -1 to -5 are indicated. ×58.—Fig. 12. Section cut 120 micra below the stem apex. The strands to presumptive leaves -1 to -5 are more developed at this level, indicating their acropetal development. ×58.

are rapidly crushed between the enlarging, dark-staining phloem cells and the lengthening, radial rows of xylem and phloem mother cells (fig. 15, 17, *obl.*). They are replaced by sieve cells developing from the radial rows of phloem mother cells (fig. 18, *ssc*). In the spring, during the period of most active development, functional phloem cells are few in number, any one cell being present for a very short time, certainly not over a few days and possibly less.

The first protoxylem element is found in any leaf primordium only after the first protophloem elements have developed into that primordium. Thus, in leaf 6 (fig. 14, *px*) one can detect the first element of the acropetally developing protoxylem on the margin of one of the procambial strands next to the pith and adjacent to the parenchyma separating this strand from its neighbor in the "double bundle". The second bundle is somewhat younger and therefore is slower in acquiring its acropetally developing xylem. Once the first protoxylem element is established, others apparently form in quick succession just centrad to the radial rows of xylem mother cells (fig. 15). The later formed xylem cells result from the progressive, centrifugal differentiation of the radial rows. Xylem cells are considered mature when they have formed lignified secondary walls and have lost their protoplasts (Esau, 1943a).

It is pertinent to point out here a seemingly close correlation between shoot elongation and vascular development. The first xylem elements formed in *Ginkgo,* more in elongating long shoots than in short shoots, are of the helical (commonly designated spiral) type (fig. 9) more rarely of the annular type. Since these elements mature during shoot elongation, they are subjected to considerable stretching, as in the two or three rows adjacent to the pith (fig. 9). Successive rows of helical elements must have formed at or during cessation of shoot elongation since they possess the close, helical thickenings and grade into circular-bordered pitted elements (fig. 9, 17). No scalariform metaxylem elements have been seen in *Ginkgo* during this investigation, a result which Bailey reported earlier (1925). However, circular-bordered pits occur in the metaxylem elements, in those transitional between protoxylem and metaxylem, and even in the protoxylem, between the turns of the helical thickenings.

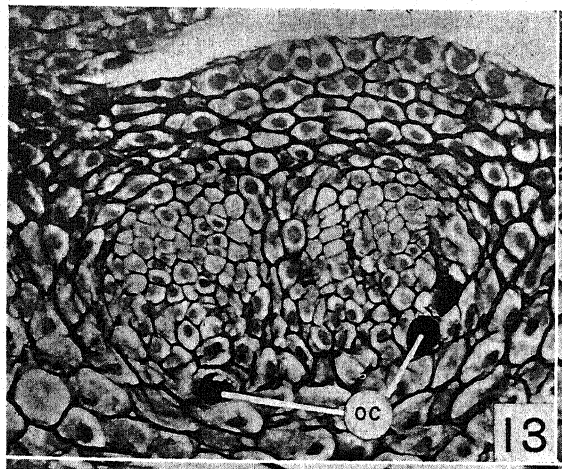
The band of cambium-like cells across the middle of each trace (fig. 15), represents the region of active, polarized, periclinal divisions, which result in the formation of both xylem and phloem mother cells. This band of cells, sooner or later, must be designated as the fascicular cambium. It is recognizably difficult in vascular plants acquiring secondary activity to determine when primary activity ceases in the development of the vascular tissues and secondary activity begins (Esau, 1943a; Bailey, 1944).

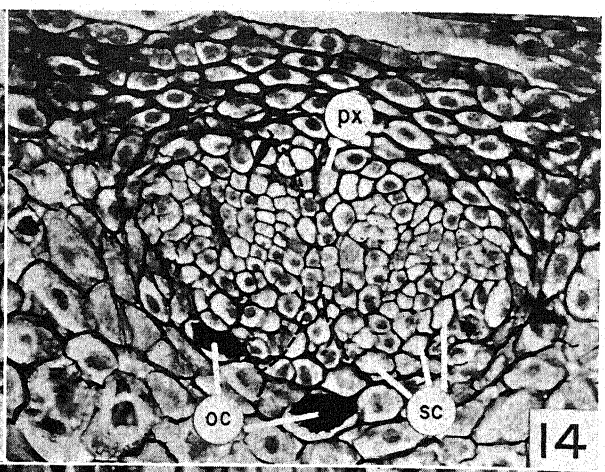
The progressive longitudinal development of the primary xylem and phloem elements was studied by the use of serial transverse sections of shoot apices. Once the phyllotaxis (Gunckel and Wetmore, 1946, Materials and Methods) and the relationship of the vascular strands to shoot organization was established (fig. 2), it was possible to follow the orderly developmental steps of xylem formation, and to a lesser extent of phloem formation, in the vascular system to any leaf.

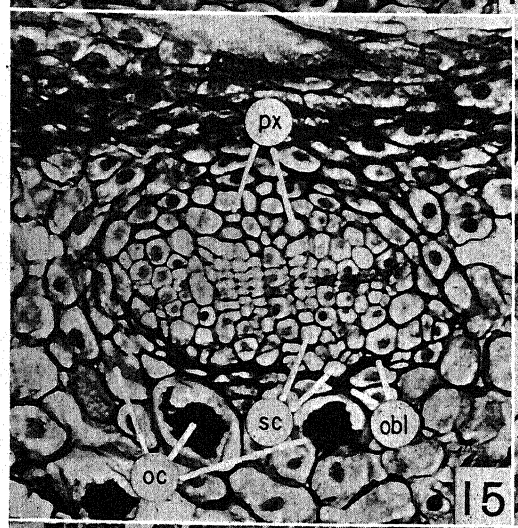
The difficulty in following the longitudinal course of protophloem development is evident if one takes into account the numerous, diverse and rapid changes just described for the phloem region in each vascular strand. Despite these changes, and also despite certain technical difficulties, it seems justifiable to predicate the continuous, acropetal development of the protophloem (table 2). It should be stated that no accurate count of mature sieve cells could be made for *Ginkgo.* Rather, the number of phloem elements, for example in leaf 6 (table 2), should be considered as an attempt to be as accurate as possible, with due recognition of: (1) the short life of each functional sieve cell; (2) the difficulty of distinguishing between mature and immature elements; and (3) the difficulty, from transverse sections, of

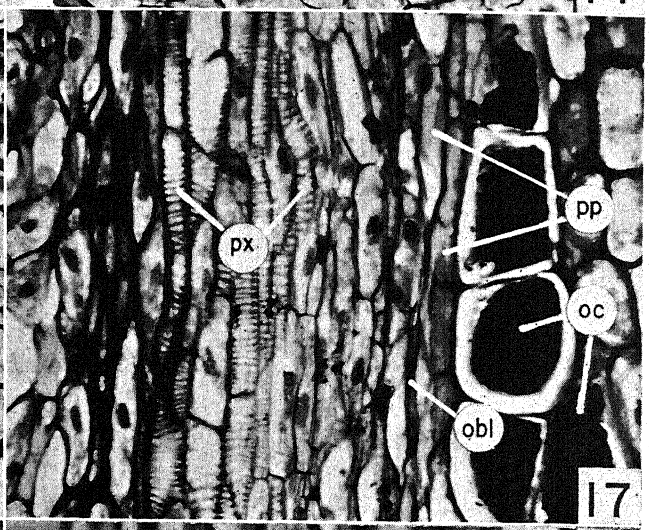
TABLE 1. *Protoxylem counts (px) for the two strands to each of the leaves 1–7 of a terminal long shoot made from a study of serial transverse sections. Where numbers are in one column, counts of separate strands were impossible because of the oblique course of strands. Material was collected when the plants were setting buds.*

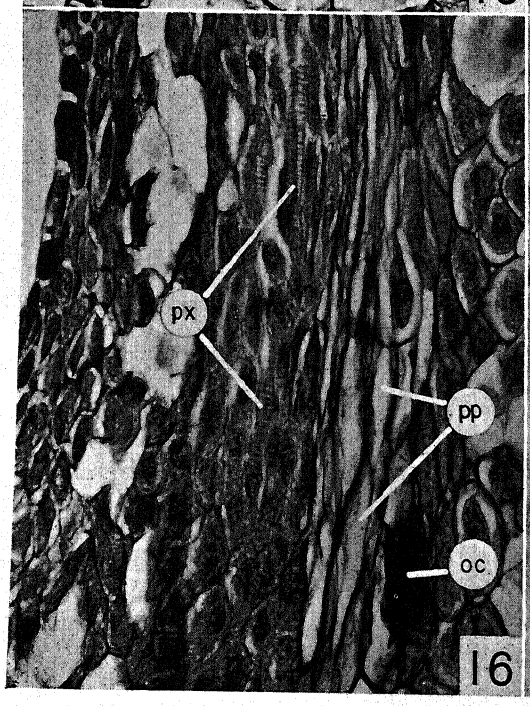
No. micra	Leaf 1 px	Leaf 2 px	Leaf 3 px	Leaf 4 px	Leaf 5 px	Leaf 6 px	Leaf 7 px
1900							1 0
1700							4 5
1500							13 13
1300							8 13
1100							7 11
900							6 7
700							8 7
500							4 6
400							2 3
300	Height 130 μ	Height 290 μ	Height 580 μ	Height 940 μ	Height 1150 μ	Height 1420 μ	2 3
200							2 2
100							1 2
80							2 3
60							1 3
40							1 2
20							4
0	Stem apex						4
−20							3
−40							1 5
−60							1 5
−80							1 6
−100						2	1 8
−150		1		5	3	3	3 9
−200		9	2 2	14	6	8	8 13
−250	0 1	18	1 3	18	9	14	.. ..
−300	2 2	20	4 7	25	19	21	.. ..
−350	5 8	19	5 15	20	27	42	.. ..
−400	.. ..	17	8 14	26	..	41	.. ..
	Confluent with strands in axis						

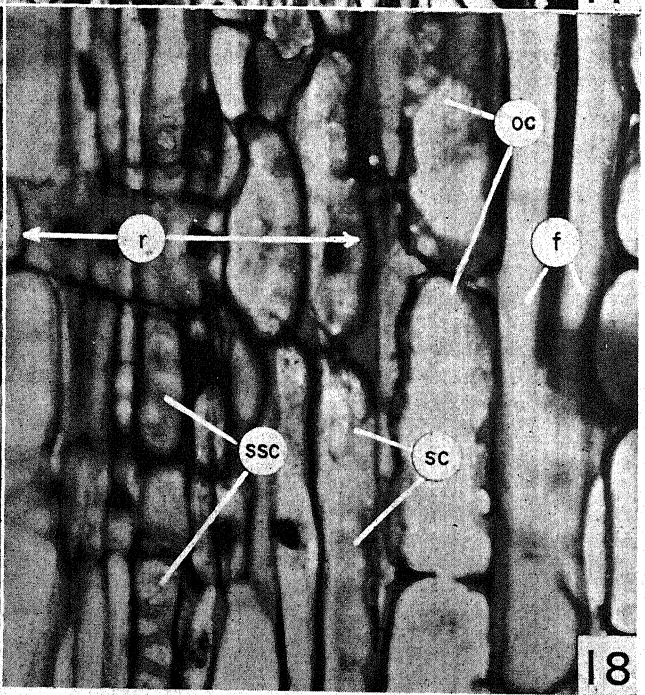

oc
13


px
oc
sc
14


px
sc
obl
oc
15


px
pp
oc
obl
17


px
pp
oc
16


oc
r
f
ssc
sc
18

following the sieve cells in the oblique course of the strands. Crushing of the sieve cells is more prevalent in the lower axis so counts of the number of elements there would be low. The obliquity of the strands in the leaf buttresses would again tend to lower the score in those regions because of the difficulty of counting elements when cut on the bias. At most these difficulties would alter quantitatively but certainly not qualitatively the trend of protophloem development. The table represents only one of many such counts but the results were comparable in the different cases.

Protoxylem development takes place acropetally only slightly behind the acropetally developing protophloem sieve cells. Thus, at about 200 micra below the shoot apex, the xylem counts in the traces of the seven youngest leaves of a long shoot (table 1) show that protoxylem elements were differentiating acropetally toward the leaf bases from the regions of those older strands which gave rise respectively to the anodic and the kathodic leaf traces concerned. The height of the first six leaves is indicated. In no case was there at this time any evidence that protoxylem had formed in the leaf blades of leaves 1–6 independently of this upward developmental trend. In older leaves, however, after the acropetally differentiating protophloem has appeared in the foliar primordia, a modification of pattern in the development of protoxylem is introduced. Leaf 6 (table 2), from a lateral short shoot apex, will serve to illustrate this situation. The counts show that protoxylem elements develop acropetally into the base of the leaf as before. Thus in traces 6a and 6b the number of mature protoxylem elements dropped to a minimum between 100 and 125 micra above the apex of the stem. Beyond this level, the figures show another increase in both traces, reaching a peak of fifteen elements per trace at about 1150 micra. Counts above this level show a reduction in elements again as one approaches the tip of the leaf. These results were confirmed by the study of many apices of both long and short shoots. The authors are convinced that, for each trace, there is a second locus of protoxylem initiation in the leaf blade following that initiated in the axis and that from this locus protoxylem then develops acropetally, and basipetally, until it becomes united with the upward developing protoxylem from below, thereby forming a continuous xylem system. However, breaks in the continuity of the xylem between that developing acropetally and the putative separate protoxylem of the leaf were not readily found until long shoots were studied during the period of elongation. Here, as might have been surmised earlier, was found the supporting evidence (table 2, leaf 4).

Discussion.—This investigation of the organization of *Ginkgo* shoots has demonstrated a close relationship between the pattern of vascular organization and the orderly appearance of foliar primordia on the vegetative apex. Evidence already provided (Gunckel and Wetmore, 1946) suggests that two acropetally developing procambial strands are already projected into the region of a presumptive leaf primordium before the primordium appears. Within each of these procambial strands, the protophloem differentiates acropetally, closely followed by protoxylem elements. As the foliar primordium develops, and the protophloem in its acropetal appearance becomes apparent in the base of the primordium, it seems clear that a second locus of protoxylem formation must be recognized in the xylem part of the foliar primordial strand. The latter protoxylem develops rapidly, apparently both acropetally and basipetally, until it joins with the protoxylem from below. This epitomy of the findings in *Ginkgo* needs comment in relation (1) to other investigations of a developmental nature on this species, and (2) to comparable investigations on other species, both gymnospermous and angiospermous.

Fig. 13–18.—Fig. 13–15. Traces of leaves 5, 6 and 8 from a single transverse section, taken at the level of the stem apex, to show progressive stages of development in successive, helically arranged leaves. These leaves are oriented so that the inner side of each leaf, subtending the apical meristem, is uppermost. All ×300.—Fig. 13. Traces of leaf 5 showing radial rows of procambial cells spreading to either flank; the phloem region is occupied by a few peripheral, very large, dark-staining cells (*oc*) and by a larger number of probably immature, protophloem sieve cells. Note that the kathodic trace (right) is more advanced than the anodic trace (left).—Fig. 14. Traces of leaf 6 showing radial rows of procambial cells extending to the flanks. Some of the protophloem sieve cells (*sc*) have lost their nuclei. The dark-staining cells (*oc*) mark the outer limits of the strands. The first mature protoxylem element (*px*) is formed in the older trace.—Fig. 15. Traces of leaf 8, showing protoxylem cells (*px*) and large, mature sieve cells (*sc*). Earlier formed sieve cells are being obliterated (*obl.*) between the large, dark-staining cells (*oc*) and the lengthening radial rows in the xylem and phloem regions. A cambium-like row of cells across the middle of each trace sooner or later becomes the fascicular cambium.—Fig. 16–18. Longitudinal sections of long shoot collected during the period of shoot elongation and showing progressive steps in phloem development.—Fig. 16. Section cut in the leaf primordium showing the elongate, mostly enucleate-celled, rather thin-walled protophloem (*pp*) sieve cells in their acropetal development. Sieve areas seen only with difficulty at this stage. The vascular strand is delimited on the outside by dark-staining cells (*oc*). A few protoxylem elements (*px*) are shown developing basipetally from the locus of xylem formation in the leaf. ×300.—Fig. 17. Section cut through leaf buttress showing leaf trace with vertical row of large, dark-staining outer cells (*oc*), with wide, protophloem sieve cells (*pp*) some still nucleate and immature and some mature—though sieve areas are not clearly defined, as their thin walls stain indifferently. Outer sieve cells show signs of crushing (*obl.*). Considerable protoxylem (*px*) is shown. ×300.—Fig. 18. Section of leaf trace showing secondary activity as indicated by a phloem ray (*r*) and secondary sieve cells with well defined sieve areas (*ssc*). The primary phloem is represented by possibly a single sieve cell (*sc*) showing sieve areas faintly; all other primary phloem has been crushed against the large peripheral cells (*oc*), here not as dark-staining as in figures 13–17, and a thick-walled fiber (*f*). ×525.

TABLE 2. *Protoxylem (px) and protophloem (pp) counts*[a] *for foliar vascular strands.—Leaf 4, from a terminal long shoot apex, selected to typify protoxylem counts during the period of shoot elongation.—Leaf 6, from a lateral short shoot apex, selected to typify the counts for older leaves during the period of winter dormancy.*

Leaf 4			Leaf 6		Leaf 6	
4a px	4b px	No. micra	6a px	6b px	6a pp	6b pp
		2350	6	9		
		2250	9	9		
		2150	12	12		
		1650	13	13		
		1150	15	15		
		1050	11	13		
		900	11	11		
0	0	750	10	9		
0	1	700	9	9		
0	1	650	8	7		
2	1	600	7	7		
3	2	500	6	5		
4	3	400	6	5		
3	4	350	7	6		
3	3	300	5	5		
2	3	200	5	5	0	0
2	3	150	7	4	0	1
3	2	140	4	4	1	2
3	2	130	2	3	2	3
2	2	120	2	3	2	4
2	2	110	1	3	4	4
2	4	100	1	4	5	5
2	3	80	2	4	5	5
2	2	60	2	2	4	9
2	2	40	2	4	4	5
2	4	20	3	3	6	6
3	3	Stem apex	5	4	5	6
2	2	−20	4	5	12	
3	3	−40	3	6	11	
1	1	−60	5	5	11	
1	1	−80	6	5	8	
1	2	−100	6	5	7	4
1	1	−120	9	9	8	7
1	0	−140	9	6	10	10
1	0	−160	10	7	13	10
1	1	−180	10	9	13	15
0	2	−200	Confluent with strands			
1	3	−220	in axis			
2	4	−240				
	4	−260				
	5	−280				

Height 1220 μ

[a] Totals are entered in single column when the oblique courses of the two strands make separate counts impossible.

Leaf arrangement in *Ginkgo* has been variously interpreted by different investigators. Nägeli (1858) described the phyllotaxis of a terminal bud as 3/8 to 5/13, Sprecher (1907) 5/13, or 2/5 to 3/8 if the axis elongates, Barthelmess (1935) a limit-divergence-angle of 137.5° or approximately 5/13. Geyler (1867) and Fankhauser (1882) described a 2/5 phyllotaxis for a mature branch. Sprecher (1907) described a 3/8 phyllotaxis for a young seedling and 3/8 to 5/13 for the terminal bud of a seedling. In explaining these varying interpretations in *Ginkgo*, Sprecher (1907) referred to the general suggestion of C. de Candolle (1895), who stated that the divergence increases as the axis grows. Van Iterson (1907) and Snow and Snow (1931) recognize similar variations in the divergence angles of diverse angiospermous seedlings as correlated with the ratio of the size of the primordium to the size of the apex. In this investigation, studies of phyllotaxis have been limited to the arrangement of leaves on shoot apices. In the two hundred or more apices investigated, the phyllotaxis in all cases was 5/13 by the system of Braun (1835) or 5 + 8 by that of the Bravais (1837); this was substantiated anatomically by studying the relationships of the leaf traces.

As Snow and Snow (1931) point out in a comprehensive review, the theories on phyllotaxis fall into two groups: (1) those in which the arrangement of leaf primordia depends upon some unknown but *internal* property or properties of the stem apex; (2) those in which the places of origin of new leaf primordia are determined by the positions of the older primordia with which they make contact; that is, their locations are determined by definite *superficial* causes or relationships.

By direct experimentation on shoot apices, the Snows (1931, 1933, 1935) concluded that each leaf arises in the first area on the stem apex that becomes both wide enough, and far enough below the growing point, to accommodate the primordium. Their findings support the belief that the determination of the locus for the origin of a new leaf primordium is dependent upon superficial factors; they thereby became supporters of the theories of Hofmeister (1868) and van Iterson (1907). It is possible that another interpretation could be placed on these experiments without contradicting in any way the constructive work of the Snows. They did not compare the internal arrangement of the cauline strands before and after a cut which isolated (1931) or divided vertically (1933) the area in which the next leaf primordium was due to arise. They did indicate that when procambial strands were severed, procambial continuity was restored, for the vascular strands were unbroken on examination. Nothing is discussed nor figured by them to preclude the possibility that the first available space for the next leaf primordium is determined as much by the space destined for the development of the procambial strands to that leaf as by the actual outlines of the previous leaves. In the regular orderly sequence of leaf development, it follows naturally that, in the pattern of the primary vascular strands, the youngest primordium would always arise in the space which is the widest and farthest below the apex (fig. 2, 11, 12). Thus, the continuous acropetal development of the procambium to the area of leaf initiation before the primordium arises, must be, if not a controlling factor in leaf arrangement, at least a corollary to the theory of phyllotaxis advanced by van Iterson and the Snows. This conclusion is in

keeping with Sterling's (1945) recent finding on *Sequoia sempervirens*. It is suggested that the disposition of new foliar primordia on the apical meristem of the *Ginkgo* shoot may well result from the correlative functioning of the apical meristem and the acropetally developing procambial strands. Unfortunately, *Ginkgo* is not the plant upon which to carry out the necessary experimental studies to test such a suggestion.

The relationship between leaf arrangement and stelar structure has been investigated by many workers. As early as 1858, Hanstein investigated leaf-arrangement in relation to the anatomy of the stem in numerous plants, mostly dicotyledonous, and concluded that the organizational pattern of the strands comprising the conducting cylinder is correlated with that of leaf arrangement. With regard to *Ginkgo,* Nägeli (1858) described a "common bundle" to the leaf, which divided into two strands a short time before it departed from the cylinder. Geyler (1867) followed the observations of Nägeli and figured the supposed condition for *Ginkgo* (fig. 3). De Bary (1884), Sprecher (1907), and Barthelmess (1935) pictured the pattern suggested by Nägeli and by Geyler.

As already indicated, the two traces of a leaf have independent origins; but it is only when they are carefully followed in serial longitudinal sections that the situation becomes evident, for the oblique course of the traces often obscures their double nature. This obliquity is especially apparent in two regions, namely, just as the leaf traces "separate" from the cylinder, and again just below the bases of the leaf primordia. However, the differential size and state of development of the two traces at any level should serve to indicate that the two leaf traces are not branches of a common cauline strand. For *Ginkgo,* at least, the origin of the "double leaf bundle" from a single strand by median parenchymatization in a radial plane, as advocated for certain species of Abies and Pinus (Chauveaud, 1904b), does not appear to be applicable, for there are two leaf traces from the beginning.

The orderly sequence of differentiation for the primary vascular elements in these leaf traces has been described. The continuous, acropetal development of protophloem is not new in gymnosperms; Crafts (1943a) noted it in *Sequoia,* and later (1943b) extended his observation to ten other species of Conifers. The same set of observations by Crafts also indicates that shortly after protophloem, in its acropetal development, reaches the leaf base, protoxylem begins to form in or near the leaf base, and develops in both directions, towards the leaf base and the leaf apex. This statement is correct for *Ginkgo* insofar as it goes; it, however, must be borne in mind that in addition to the protoxylem formed in the procambium of the leaf primordium, an earlier center of differentiation of protoxylem exists, for protoxylem extends acropetally to the leaf traces from the cylinder below even as did the protophloem, though somewhat behind the latter. This acropetal development of protoxylem from strands below has not, so far as the authors know, been described for gymnosperms, though it has been noted several times for angiosperms (Esau, 1943a).

Sprecher (1907) has followed the progressive development of primary xylem and phloem in the vascular strands of *Ginkgo.* He described the first differential appearance in the phloem as of large cells, poor in protoplasm, and for the most part without nuclei. What he describes as tannin cells—the dark-staining cells of the present authors—were usually formed on the outside of the strand. The protophloem might become thick-walled and later fibrous and a "generative" layer appear between the xylem and phloem. That protophloem, which did not become fibrous, was crushed against the tannin cells. The mature protoxylem appeared after the protophloem, first as spiral tracheids and later as net-like and circular-bordered pitted tracheids. He also noted that the wood in a short shoot is less dense, with more frequent rays; fibers are less frequent in the phloem of the short shoot, but there were many more crystal-bearing cells in both phloem and pith.

The present study supports the major part of Sprecher's findings. Apparently, Sprecher considered the peripheral, large protophloem elements as functional; though he states that some of them have nuclei, he does not mention these as functionally immature. He makes no mention of sieve areas on any of these elements.

The formation of phloem elements in the stem of *Ginkgo* has also been studied by Léger (1897). He described what he called *secretory* tubes on the periphery of the strand—the dark-staining cells of the present authors—and considered that they were derived from procambium, a fact confirmed by the present study. He reported the nacreous walls of the large, peripheral, phloem cells, suggesting their sieve cell nature; these were, however, short-lived, being rapidly crushed against the secretory tubes. Léger does not refer to sieve areas on these early protophloem elements. He considered the later phloem elements with nacreous walls, which developed in radial rows, as of secondary origin. New secretory tubes were formed centrad to the original tubes but peripheral to the radial rows. Some of the original, outer tubes become fibrous, others develop crystals. Léger's results agree generally with the present findings, except that the early-formed radial rows of phloem cells are not considered of secondary origin, nor do the secretory cells form tubes or canals. The present authors find that the crushing of the protophloem, and also of the later, mature sieve cells occurs very rapidly so that the life of any one cell is only a matter of a few days or even less and only a few functional cells are present at any one time. It was not possible to distinguish between the relative effects of the elongation in the long shoot and the radial expansion in the short shoot on the obliteration of the early phloem.

In his study of certain coniferous seedlings,

Chauveaud (1902a, 1902b, 1904a) called attention to the presence in the hypocotyls, roots and cotyledons of what he termed collectively phloem precursors, the individual elements being *precursors of sieve tubes*. These cells precede the regular, primary sieve cells. They are characterized by wide-diameters and initially thin walls. Later these walls progressively thicken, the outer, first-formed elements developing sieve areas on the proximal or inner wall while later precursor elements have them on all walls. The early sieve cells of *Ginkgo*, quickly crushed, clearly conform in position and in nature to the precursor sieve tubes of Chauveaud except that they occur throughout the shoot system. A study of roots has not been made. The authors see no reason to distinguish between sieve cells and precursors; the differences seem to be those of degree rather than of nature (Abbe and Crafts, 1939; Esau, 1943a).

In this study, a distinction is drawn between protoxylem and metaxylem (Bugnon, 1924; Esau, 1943a). The protoxylem is considered to be the early differentiated primary xylem, usually characterized histologically by annular or helical thickenings (Russow, 1872, 1875). It is ordinarily found in elongating parts of vascular plants (Russow, 1875; Frey-Wyssling, 1940; Goodwin, 1942; etc.). By contrast, the metaxylem is conceived as that later-formed primary xylem, differentiated as elongation ceases or after it has stopped (Frey-Wyssling, 1940; Goodwin, 1942) and is more often characterized by scalariform, reticulate, or circular-bordered pitted elements. Whether there are causal relations between wall thickenings in the primary xylem and elongation is not clear at the present time. Certainly it is difficult to set any finite definition for distinguishing between late-formed protoxylem and early-formed metaxylem.

In *Ginkgo*, it is quite possible to recognize early protoxylem, and, as earlier pointed out, the late circular-bordered pitted metaxylem. The transitional elements of close helices bearing occasional circular-bordered pits, and of reticulate elements with circular-bordered pits, are debatably classified. As Bailey (1944) points out, "Not until extensive data are available from a wide range of vascular plants, including structurally primitive as well as highly specialized representatives, can one determine for example, whether such morphological concepts as protoxylem and metaxylem should be redefined and clarified (Frey-Wyssling, 1940; Esau, 1943a) or abandoned (Popham, 1940)."

SUMMARY

A close correlation exists in *Ginkgo biloba* between the pattern of vascular organization and the orderly appearance of foliar primordia on the vegetative axis. Evidence is provided which suggests that the acropetal development of the procambium to the area of leaf initiation before the primordium appears must be, if not a controlling factor in leaf arrangement, at least a corollary to the theory of phyllotaxis.

The primary vascular system is a sympodial network. The two traces to any one leaf are morphologically distinct in their origin and in their development, the one arising from a vascular strand to the next older leaf in the same "spiral" series, the other from a strand in an adjacent "spiral". This interpretation is in contrast to the classical concepts of Nägeli and Geyler.

Within the procambial strands, as foliar primordia develop, protophloem elements are differentiated acropetally. The cells in the outer phloem region enlarge. Some of these become dark-staining short cells, some elongate fibrous cells, some crystal-bearing cells, while others in the same region go through the characteristic changes leading to sieve cell formation.

This acropetal development of protophloem sieve cells is closely followed by acropetally developing protoxylem elements. Shortly after the appearance of the first sieve cells in a leaf primordium, a second locus of protoxylem formation can be recognized in the xylem part of the strand within the primordium. This protoxylem develops acropetally into the growing foliar primordium, and basipetally until it joins with the protoxylem from below.

Later formed protophloem and protoxylem conducting elements are differentiated in the radial rows of cells which develop in each bundle from polarized periclinal divisions. The protophloem sieve cells prove to be of very short life. The first protoxylem elements are helical or annular. They grade into helically pitted elements which are transitional to metaxylem elements of circular-bordered pitted type.

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DIFFERENTIATION AND PATTERN IN MONSTERA DELICIOSA. THE IDIOBLASTIC DEVELOPMENT OF THE TRICHOSCLEREIDS IN THE AIR ROOT [1]

Robert Bloch

To the student of both normal and abnormal cell behavior anything which can be learned about conditions under which cells differentiate (develop different properties during ontogenesis) is of theoretical and practical consequence. At the plant growing point embryonic cells in all likelihood start out as morphologically very similar and genetically identical totipotent units, although probably no two cells in the organism remain exactly alike for long; very soon cells can be distinguished which exhibit specialized combinations of various cellular properties and activities (as to growth, membrane structure, contents, and physiological character). Usually such cells differentiate in association with the formation of specific tissues in typical locations within the plant organ; sometimes, however, they are arranged in less regular fashion, namely, as individuals scattered through the ground tissue, to which Sachs applied the term "idioblasts." Among the latter those cells appear of special interest which occur as solitary, actively proliferating elements among the more static and regularly shaped elements of the fundamental tissue. Notable among these cells, which often combine developmental and structural features of bast fibers, sclerotic parenchyma cells, hairs and other forms (for classification see Tschirch, 1885; Foster, 1944), are certain actively growing, prosenchymatous and branched sclereids which ramify between elements of the ground tissue, such as the well-known astrosclereids of leaves (de Bary, 1884; Solereder, 1908; Foster, 1944). In a number of plants these cells are known to develop by proliferation of internal initials abutting on the intercellular space system, as in the *Nymphaeaceae* and *Menyanthoideae* (Gürtler, 1905), the *Araceae* (van Tieghem, 1866), and *Rhizophora* (de Bary, 1884; Gürtler, 1905); where the lacunae are large, the development of these cells reminds one of that of external hairs from an epidermal surface. The terms "internal hairs" (Meyen, 1837) and "trichoblasts" (Sachs, 1882; Gürtler, 1905) applied to these cells refers to this property; they are also frequently described, together with other kinds of idioblastic sclereids, as "spicular cells" (Solereder, 1908). Both the terms trichoblast and spicular cell are also used, however, to denote other kinds of plant cells; the present writer, therefore, proposes the use of the term *trichosclereid* for such forms as are described in this paper. These cells can be distinguished clearly both morphologically and developmentally from the brachysclereids which are differentiated in the hypodermal region of the cortex of *Monstera* air roots during later development (Bloch, 1944).

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Comparatively little is known about the induction and development of trichosclereids and other forms of idioblastic sclereids. Gürtler (1905) studied their development in *Nymphaeaceae* and other plants; Foster (1944, 1945) has recently investigated the development of sclereids in compact tissue of the petiole of *Camellia japonica*, as well as in the more highly lacunate tissue of the lamina and petiole of *Trochodendron araloides*. Characteristically in all cases the idioblasts developed from small initials which, however, did not occur in any definite pattern; in *Trochodendron*, according to Foster, all spongy parenchyma cells retain the capacity to form sclereid initials. The most outstanding feature of the sclereids in *Camellia* and *Trochodendron* appears to be their capacity to grow under different intercellular conditions. Thus in *Camellia*, cell processes intrude between densely arranged collenchyma or parenchyma cells and cease growth when a large space is entered, while in *Trochodendron*, where sclereid formation occurs over a relatively long period of time, the branches of the sclereids grow through spaces and intervening tissue alike. The potency here of considerable portions of the primary wall to grow out would appear as a property which these cells share with those of the ordinary parenchymatous type which retain the capacity for wall growth for considerable time. It appears significant that in *Camellia*, where the growth is almost entirely intercellular, but in close contact with the parenchyma walls, pit connections are apparently re-established between the walls of parenchyma cells and sclereids, much as in other intrusively growing fibers, while in *Trochodendron* pits are restricted to the central part of the sclereid which remained in contact with parenchyma cells.

The present writer has worked with the long, pointed, fibrous and more or less branched cells which ramify in air spaces of various tissues of *Araceae*, especially the *Monsteroideae*. These have been described several times on account of their very peculiar and variable forms (van Tieghem, 1866; Wiesner, 1875; Lierau, 1888; Gürtler, 1905; Solereder, 1919; Solereder and Meyer, 1928), but relatively little is known about their development. Van Tieghem (1866) mentions their origin from small, parenchymatous initials which form processes into the adjacent intercellular space system. These processes may be seen readily enough in transverse sections through mature regions of the mesophyll of leaves or the cortex of roots and air roots (fig. 8); the early origin of the initials, however, requires more intensive study. The author noticed previously (1944) that in the air root of *Monstera deliciosa* the initials of these sclereids appear very early near the

apex of the root meristem, and that these initials send out fast growing processes which grow upward and downward between the parenchyma cells. The tissue in which they arise is a rib meristem of considerable symmetry, and such tissue would appear to have certain natural advantages for the study of early intercellular relationships in idioblastic development.

MATERIAL AND TECHNIQUE.—Tips and mature parts of air roots of *Monstera deliciosa* were killed in C R A F and the development of trichosclereids studied from the youngest stages onward, both in longitudinal and transverse sections, 12–20 μ in thickness, stained with safranin and fast green. Air roots of various sizes, from 1.5 to 15 mm. diameter and of varying length, were studied. Young stages of trichosclereids in hand sections of fresh material may be profitably studied after staining of the young cell walls and cytoplasm with ruthenium red. The form and length of fully grown sclereids were studied after maceration and mechanical isolation.

THE DEVELOPMENT OF THE MOTHER TISSUE AND INTERCELLULAR SPACE SYSTEM.—The development of the sclereids is so closely related to changes in the cortical parenchyma that it is advisable to describe these first. Both transverse and longitudinal sections were studied. At a distance of about 700 μ from the apex of the cone of the meristem the cortical parenchyma cells have begun to vacuolate, and show in longitudinal sections the typical configuration of a rib meristem. In it numerous files of cells may be distinguished, each of which is made up of the derivatives of one meristematic cell of the cortex, now consisting of 3–5 cells (fig. 3). It will be noted that these files are not always exactly continuous, but occasionally stagger or alternate somewhat; as the cells vacuolate and expand longitudinally, however, this discontinuity becomes less distinct.

The intercellular space system begins to develop very early and may be recognized in both longitudinal and transverse sections. A number of channels may be seen to run longitudinally between the files to within a short distance of the apex, and in the longitudinal sections small intercellular spaces may also be seen at the corners where the cell files abut on each other (fig. 3). Both the somewhat tapering shape of each file and the presence of air spaces where the files abut on each other, make it easy to recognize the boundaries of the original cortex cells.

Transverse sections show that the air space systems undergo considerable changes during ontogenesis. Already near the apex three kinds of spaces may be distinguished: triangular, somewhat larger quadrangular, and still larger pentangular spaces (fig. 1 a–c, 2). The number of air channels with more than three sides increases as vacuolation and differentiation of the parenchyma proceed, and is due to various factors, namely (1) some cell divisions which meet an air space (fig. 1 a); (2) cell divisions in opposite position (fig. 1 b); and (3) particularly early and later loss of contacts between cells (fig. 1 c–d). The air spaces finally become much larger than the diameter of the trichome-like sclereid processes which originally entered them (fig. 1 d, 8), and in the case of later loss of contacts several air spaces containing sclereid branches may coalesce so that finally several branches which belong to different sclereids are seen in the same space (fig. 1 d).

INITIATION OF TRICHOSCLEREIDS AND THEIR GROWTH IN RELATION TO THE INTERCELLULAR SPACE SYSTEM.—In the cortex of young air roots, varying in diameter from 1.5 to 3.0 mm., polar, differential divisions occur, often as near as 700 μ from the apex. These divisions cut off one small cell, and very frequently a pair, at the basal (proximal pole) in many, but not in all cell files (fig. 4, 5). In this manner a distinct difference in cell size results. These last formed small cells may be readily recognized, on account of their dense and more deeply staining cytoplasmic content and their nuclei which are relatively large in relation to the cell volume (fig. 4, 5). In roots with comparatively little meristematic activity often only one small cell is formed in one cell file.

The small cell located at the basal (proximal) end of the cell file becomes a trichosclereid initial or mother cell from which processes develop into the adjacent parenchyma. Generally this cell is the only one which develops into a trichosclereid. If there is a second small cell present, it usually expands somewhat longitudinally, like the cells of the ordinary parenchyma; sometimes, however, both cells develop into trichosclereids (fig. 9). Thus both relative size, resulting from an unequal, differential division, and relative *position* within a cell file characterize the sclereid initials and make it easy to locate them at their earliest stage, i.e., when the cortical mother cells begin to divide. The foliar sclereids of *Camellia* and *Trochodendron,* according to Foster (1944, 1945), can be recognized only by the larger size of their nuclei, but in the present case a more determined type of idioblastic development occurs, from divisions which are both differential and bear a constant positional relation to the cells of the mother tissue.

The sclereid initials abut on several longitudinal air channels, usually one of them being a larger four- or five-sided space (fig. 2, 4, 8). After very little longitudinal expansion of the initial, tubular processes begin to grow out from it which enter the four-sided intercellular channel (fig. 2, 6). At this stage the growth of the invading branch thus appears free, like a root hair, or a tylosis pushing into a vessel. The originally small branches grow with great rapidity and also expand somewhat and thus often reach the end of an intercellular channel (fig. 7). Throughout their growth the trichosclereids are thus characterized by the fact that they closely follow the developing intercellular system and by their ability to change their direction of growth, to branch and to fork at their ends when obstacles of various kinds are encountered. In this manner forms are produced which closely resemble certain fibers, such as those found in *Cannabis* and *Luffa* (Sinnott and

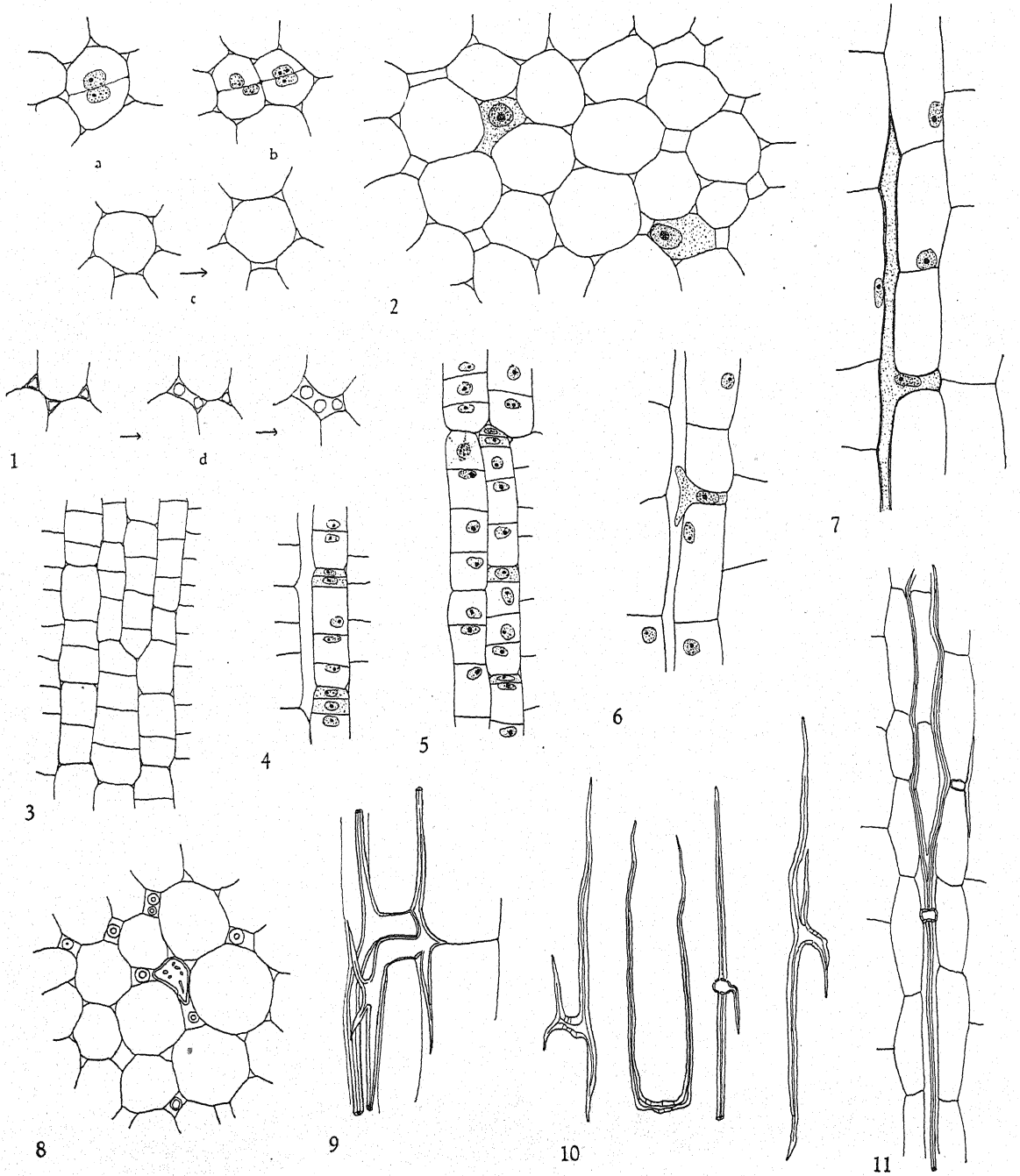


Fig. 1–11.—Fig. 1. Transverse sections through cortical parenchyma of air root, showing origin of quadrangular and pentangular air spaces by early cell divisions (a and b), by early loss of cell contacts (c), and by later loss of cell contacts (d).—Fig. 2. Transverse section through cortical pa enchyma, showing two trichosclereid initials, one of which sends out a process into a quadrangular air space. ×450.—Fig. 3. Longitudinal section through root meristem 500 μ from apex, showing files of cells. ×240. (In all longitudinal sections the root apex is toward the bottom of the page.)—Fig. 4. Similar section, showing formation of trichosclereid initials at basal (proximal) end of two cell files abutting on longitudinal intercellular space. 700 μ from apex. ×240.—Fig. 5. Similar section; one nucleus (upper left) is in prophase. ×240.—Fig. 6. Similar section, 2200 μ from apex, showing the formation of processes into an intercellular space (compare fig. 2). ×240.—Fig. 7. More advanced stage, in which the basal end of the process has reached the end of an intercellular space; 2200 μ from apex. ×240.—Fig. 8. Transverse section similar to figure 2, showing both the initial part of the trichosclereid and several processes in the thickened mature stage. The initial part is pitted. ×195.—Fig. 9. Longitudinal section, showing parts of two mature trichosclereids which have developed from divisions as in figure 4. ×240.—Fig. 10. Four comparatively short trichosclereids from macerated material, showing diversity of branching and distribution of pits; the walls are somewhat swollen. ×105.—Fig. 11. Trichosclereid, the upper process of which has formed two branches around a parenchyma cell. ×105.

Bloch, 1943), although these grow in much closer morphological and physiological contact with the adjacent parenchyma cell walls. The type of growth observed in the *Monstera* trichosclereids appears to be that formerly described as "intrusive," in which growth occurs mainly at the tips of proliferating portions of the cells, with no appreciable shifting or sliding of larger portions of cell walls (Sinnott and Bloch, 1939, 1943). In the inner cortical regions trichosclereids occur whose processes follow closely the course of the intercellular canals in zig-zag fashion (fig. 12). The diameter of these intercellular channels changes at regular intervals owing to the curved surface of the adjacent longitudinal parenchyma walls, and the trichosclereid processes show corresponding wider portions. It was found that this relation had not changed after maturation and sclerification of the processes (fig. 11, 12) which would indicate that there had been no slipping or sliding of large portions of the processes within the intercellular canal, any possible sliding occurring only at the tip. Furthermore, small lateral branches and outgrowths, which protrude into the adjacent parenchyma, are never subsequently torn out of place.

The growth of the sclereid processes is free within an intercellular space or in rather close contact with parenchyma membranes, but wherever an obstacle in the form of a cell wall is encountered, the processes are easily deformed and deflected from their course. In the early stages the advancing arms can frequently be seen in close contact with the middle lamella (intercellular substance) of the parenchyma cells at the end of an intercellular space (fig. 7), and in fully differentiated trichosclereids the very narrow and pointed tips of long processes may occasionally be seen in close contact with the parenchyma walls. It is difficult, of course, to say whether and to what degree the sclereid might actively contribute to the separation of the primary membranes of the cortex cells. In *Monstera* this process seems to take place relatively easily, and the intrusive growth of the processes may be considerably facilitated by the fact that it proceeds along the course of natural air space formation. The wall of the processes seems never to be in very intimate connection with the parenchyma walls since it separates most readily under various treatments.

Branching of the trichosclereid may be associated with the formation of new air spaces adjacent to it, or may be induced by obstacles encountered by the processes, such as cell contacts, or by growing parts of other trichosclereids. Mostly two or four processes are seen to develop from one initial (fig. 10); H-shaped cells, known to occur in many species, are common (fig. 15). Branching and bending of processes around intervening cells are frequent (fig. 11), and very irregular forms were figured by van Tieghem (1866) from the leaves of *Spathiphyllum lanceaefolium* and *Tornelia fragrans*. The tip of the growing sclereid process may be induced to branch by even a slight obstacle such as the young tip of another process, growing in the same air space, but in the opposite direction (fig. 14). Branching of processes within an air space, however, does not seem to occur as readily as in other types of freely growing hairs, for example in some of the internal hair cells described by Gürtler (1905).

Sclereid initials may remain dormant for some time until suitable conditions for their further development arise. They then may grow into a space full of other more mature sclereid processes.

SECONDARY THICKENING OF THE TRICHOSCLEREIDS.—In the mature stage the walls of the sclereid arms are considerably thickened and the lumina reduced; the cells possess pointed, tapering tips, much like bast fibers, and the thickness of the secondary layers decreases gradually toward both the tip and the initial (fig. 13, left). The basal or central initial portion of the cell is usually less thickened (fig. 13, 16), a fact already mentioned by Wiesner (1875) for sclereids of the mesophyll. The same author noted an irregular thickening of the wall the external contour of which, similar to the bast fiber of jute, is not always parallel to the inner one. In the present investigation the same irregularities were noted. Wiesner also found that the secondary wall of the arms shows some spiral striation, but no pits. In the present study it was found that pits occur in the initial portion of the cell which contains the nucleus, but no pits were found in the branches. Obviously the initial part of the cell retains from the beginning close morphological and physiological contact with the neighboring cells, similar to the sclereid of *Trochodendron*.

As the air spaces grow wider, the growth in width of the processes does not keep pace with them. In *Raphidophora decursiva,* another species of the *Monsteraceae,* however, the branches fill almost the entire space, even in the mature, sclerotic stage, showing the imprint of the outlines of the parenchyma on their walls (fig. 17). Van Tieghem noted the same in *Raphidophora pinnata* (1866; p. 153).

The mature walls of the *Monstera* sclereids are impregnated with suberin, but not truly lignified. They stain readily with Sudan III and safranin, but do not give the lignin reaction with phloroglucin and hydrochloric acid, unlike the sclereids in other members of the *Araceae*.

Final thickening and suberization of the wall do not take place until a considerable distance from the root tip. This distance varies with the type of air root and with its diameter; in a thick air root, 80 cm. long and 15 mm. wide at its base, the sclereids at 20 mm. from the tip and 7 mm. diameter were still thin-walled.

Striking differences in the onset of wall thickening and chemical impregnation occur, not only between individual sclereids, but also between the various parts of the same cell. At the same root level sclereids are found which are fully mature, while others may be still thin-walled or occasionally even beginning to develop (fig. 13). Furthermore, parts of the long arms of one cell, often the tip or a portion some

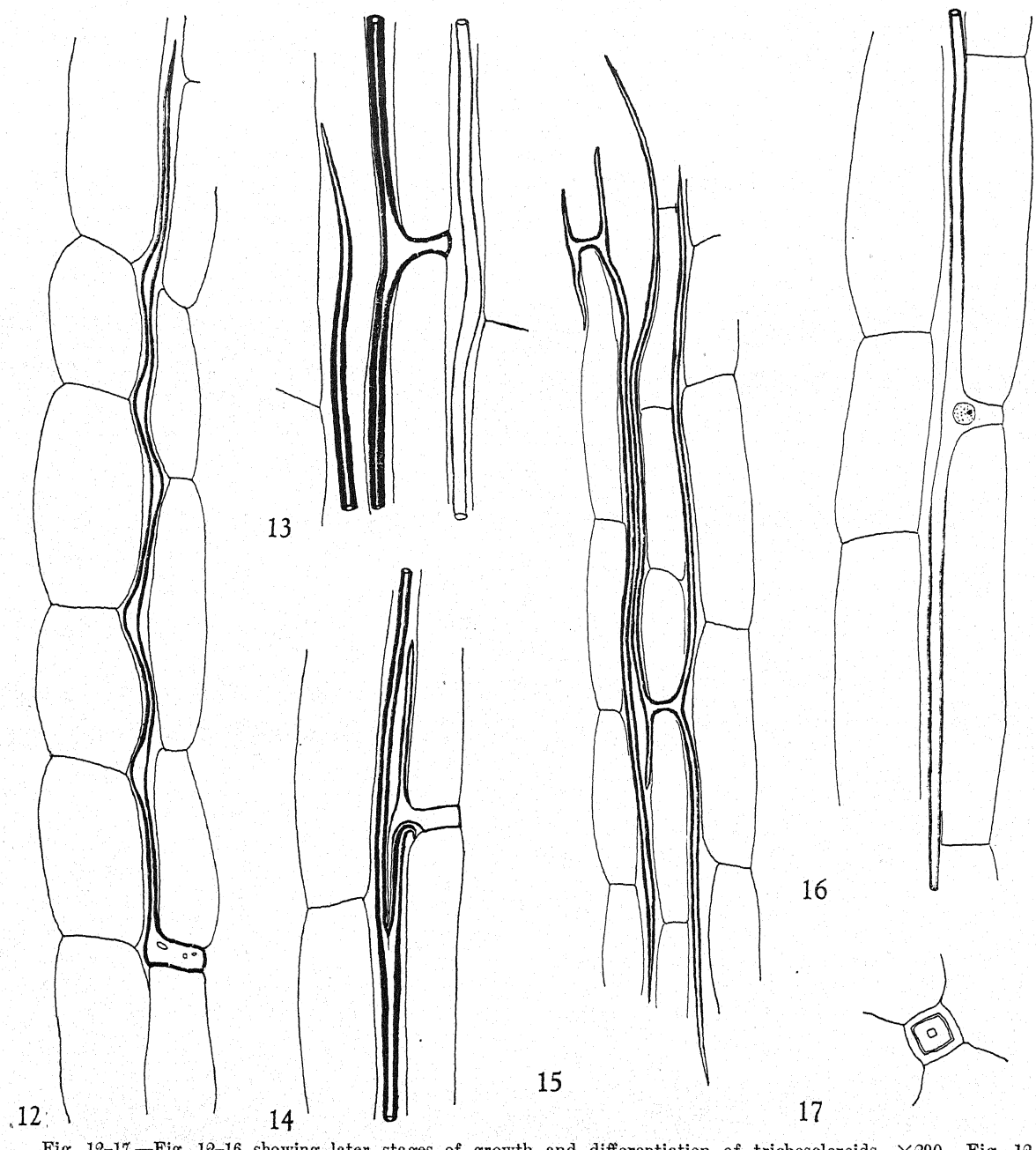


Fig. 12–17.—Fig. 12–16 showing later stages of growth and differentiation of trichosclereids. ×290.—Fig. 12. Trichosclereid, showing relation between regions of lateral expansion in process and width of intercellular canal.—Fig. 13. Three trichosclereids which have developed close to each other, the two at left are fully differentiated, the one at right still thin-walled. Note that the secondary layers of the wall become thinner toward the tip and toward the initial part of the cell.—Fig. 14. Two trichosclereids in the same intercellular canal, one process grows upward and has split in two where it encountered the small process growing downward.—Fig. 15. Two H-shaped sclereids.—Fig. 16. Trichosclereid showing beginning of secondary thickening of the wall. The central initial portion is still thin-walled and shows the nucleus.—Fig. 17. Transverse section through process of mature trichosclereid is air root of *Raphidophora decursiva,* showing collenchymatous thickening of parenchyma cells around original air space, quadrangular trichosclereid process which fills the air space completely, and small quadrangular lumen of process. ×360.

distance from it, may begin to thicken and change chemically, while other parts are still thin-walled, and this may account for the subsequent variation in the thickness of the secondary layers at maturity which were noted by Wiesner and the writer. In fibers, such as those of *Luffa,* the growing tip is generally the last to lignify; in the present case the central, initial parts and adjacent areas of the branches mature last (fig. 16).

The diameter and lumen of the trichosclereid

arms may also vary considerably, not so much because of differences in the thickness of the secondary layers, but on account of the previously described expansion of the processes in regions where the intercellular channels naturally widen. Especially in the inner cortical region this occurs frequently, and a peculiar zig-zag-shaped course of the branches results. In the regions where these expand laterally the secondary layers are frequently, but not always, somewhat thinner (fig. 12).

All these observations seem to indicate that both growth and differentiation are more variable and irregular in sclereids than in fibers, bast cells, parenchyma cells, etc. The process of wall differentiation seems not to be entirely controlled by a gradient which affects all cells at the same level evenly, but rather to be an individual reaction of the cell in relation to local conditions. Such conditions must vary considerably, since the cells are of considerable length; cells 2–3.5 mm. long are not rare. Environmental conditions would thus determine to some degree not only the growth reactions in various parts of the same cell but also the ultimate differentiation of its wall.

Discussion.—The trichosclereids in the air root of *Monstera deliciosa* are interesting in several respects. The most striking feature is their origin from mother cells near the apex, formed by late, polar and differential divisions, analogous to those occurring in the hypodermis, at the basal ends of cell files of the cortical parenchyma. The position and origin of these "idioblasts" are thus not accidental, random ones, but have a definite relationship to the cell pattern in the mother tissue from which they arise. A difference between sclereid initial and ordinary parenchyma in cell size and in the relation of the nucleus to the cell volume results, and highly embryonic initials are formed adjacent to cells somewhat more advanced in maturation, comparable to the trichoblasts of the surface layer of grass roots, which also form a characteristic pattern in the mother tissue. The root hair cell, however, later undergoes considerable longitudinal expansion, but the sclereid initial grows very little, its expansion being limited almost entirely to the tubular branches. Developmentally the trichosclereids of *Monstera* can best be understood if one remembers that they are formed as densely cytoplasmic cells at a time when their neighbors are already in the vacuolating and expanding stage. Like the trichoblasts of the epidermis they are located on surfaces abutting on air, under conditions which apparently favor specific expansion, growth and subsequent differentiation of the wall.

Compared with the other cells of the cortical parenchyma the development of the trichosclereids would seem to indicate a highly specific nature as a result of differential reactivity. A similar difference in behavior occurs between the root hair cells (trichoblasts) and the ordinary cells in the surface layer of roots, but in this case the distinction is frequently not very sharp, and ordinary epidermis cells may be induced to form root hairs also. In the present case it was never observed that other cortical cells were capable of developing into a trichosclereid, while previous regeneration experiments showed (Bloch, 1944) that, at a later stage of general differentiation and developmental potency of the ground tissue, any cortical parenchyma cell in the air root of *Monstera* becomes able to differentiate into a hypodermal brachysclereid, if the conditions for their differentiation are experimentally created which in a certain position exist adjacent to an external or internal surface.

In other cases of idioblastic development of sclereids similar patterns and intercellular relationships may exist, but may not always be as pronounced and easily recognizable as in the case here described. Foster (1945) in his description of sclereid development in the spongy parenchyma of *Trochodendron* has emphasized that all parenchyma cells appear to retain the capacity to enlarge and to ramify, and that "the entire career of development of an idioblastic sclereid is marked by a 'rugged individualism' which suggests that the normal correlative forces operative during the differentiation *en masse* of parenchyma elements have been replaced locally by new and unique factors." It is significant in this connection that the definition of the term "idioblast" used by Sachs and other authors for these and similar cells does not refer to any rules or factors which might control their distribution in the mother tissue. Their scattered distribution makes them often appear less typical a part of the general tissue pattern than other more compact tissue systems. Adding to this feature the characteristic "invasive" or rather long-continued character of their growth, which distinguishes them so sharply from their neighbors, the impression may be gained that these cells possess capacities specifically different from the majority of plant cells in closely correlated cell aggregates. However, the distribution and development of the idioblastic trichosclereids of *Monstera,* which have a close genetic relationship to a pattern of cell divisions in the mother tissue, would appear to be as much under organized control as other types of solitary cells, such as stoma cells, certain external hair cells, and other forms which have not been considered to be of idioblastic nature, since more was known about their developmental history. In the growth of the trichosclereids no phenomena were observed which would be fundamentally different from those already known to occur generally in the growth of plant cells, various methods and possibilities of which have been discussed in previous papers (Sinnott and Bloch, 1939, 1943). It is known that many plant cells are able to grow in several ways in response to changing external conditions. A parenchyma cell may show very restrained growth in most types of tissue, under certain conditions its membrane may exhibit considerable differential growth, or may grow freely, for example as a tylosis, into an adjacent lacuna. The trichosclereids of the *Monstera* air roots arise from densely cytoplasmic initials

formed in the meristem by relatively late divisions and in a position abutting on lacunae which are already present or fast developing. These facts are obviously related to the subsequent growth of the sclereids, which is not shared by their neighbors, and should be taken into account in its further analysis. Such "individualistic" behavior, especially emphasized by Foster (1945), appears as a natural concomitant in this extreme type of differentiation in which cells in different stages of development and therefore of different reactivity and developmental potency are exposed to probably very similar, if not the same, external conditions. It is not, in the writer's opinion, an instance of morphogenetically uncontrolled, independent, or pathological growth. The phenomenon of idioblastic development of this kind in plants rather appears as part of the more general problem of differentiation and pattern.

All histological differentiation can be interpreted as a process in which cells become individually different from each other. During this process they go through different stages of reactivity or developmental potency (Bloch, 1944) upon which varied local conditions have differential ways of "control"; thus many individual characteristics are developed. How this is achieved in a specifically and well coordinated manner within the pattern of a given genotype is a major problem of organized development.

The trichosclereids in the air root of *Monstera* offer still another interesting aspect. It has been suggested that their function may be a mechanical one, somewhat comparable to a network of fibers. It appears of interest to compare the pattern in *Monstera* with the mechanical system in the air roots of some orchids, for example *Cattleya,* the so-called "Réseau de Soutien." While in the cortex of air roots of *Monstera* and other Araceae a mechanical system is achieved by a formation of a pattern of fiber-like, sclerotic hairs, in the "Réseau de Soutien" of the cortex of orchid air roots, a continuous network is formed in a whole group of cells by branching bands of thickened and lignified walls, comparable somewhat to the Casparian strip in the endodermis or the thickening in xylem elements (Sinnott and Bloch, 1945). One natural consequence of these differences is that in the air root of *Monstera* a branch root could readily make its way through the cortex, simply pushing aside the sclerotic processes which form no continuous mechanical system and therefore offer little resistance. In the air root of orchids, however, the continuous network would form a real obstacle, and it was found (Bloch, 1935) that here sectors of the root in which branch roots are being formed from the beginning remain free of the lignified thickenings.

SUMMARY

In the air root of *Monstera deliciosa* trichosclereids are formed early in the cortical rib meristem, which proliferate between parenchyma cells like hairs, sending out long processes which are prosenchymatous like those of astrosclereids or fibers. The cells are thus clearly distinguished from the brachysclereids of the same root which differentiate at a much later stage from non-specialized cortical ground parenchyma cells of the hypodermal region or adjacent to internal surfaces.

The trichosclereids originate from late, differential, polar divisions of cortex cells near the root apex, analogous to those which give rise to passage cells in the hypodermis. These special divisions occur at the basal (proximal) ends of cell files in the rib meristem, and the trichosclereid initials can be recognized by their position, small size, dense content and large nucleus. Their subsequent specific activity, which distinguishes them from their differently reactive neighbors, consists in the formation of prosenchymatous processes which grow rapidly into the intercellular space system as it develops between the cortical parenchyma cells. This behavior appears to be correlated with their late appearance as "embryonic" cells and with the presence of air spaces. Their subsequently long-continued growth is comparable to that of the external trichoblasts of the grass root epidermis and other plants. The distribution of trichosclereids in the air root of *Monstera* can thus be traced back to a pattern of differential cell divisions in the fundamental tissue which precedes it, and later idioblastic development appears as the outcome of specific differentiation rather than uncontrolled growth of solitary cells.

The growth of the processes of the trichosclereids resembles that of other intrusively growing fibers and sclereids and bears a close relationship to changes in the intercellular space system; later differentiation of the wall of the sclereids is comparable to that of ordinary fibers or sclerenchyma cells, although somewhat more irregular. Pit canals occur only in the initial, central portion of the sclereid, and secondary thickening and suberization are not always initiated uniformly over the entire length of the long tubular branches of a sclereid, but are apparently often subject to variable local conditions.

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GROWTH OF TOMATO ROOTS AS INFLUENCED BY OXYGEN IN THE NUTRIENT SOLUTION [1]

Louis C. Erickson

THE GROWTH of many species of plants in water culture is greatly improved by bubbling compressed air through the nutrient solution. This response suggests that the same factors important in soil aeration, *i.e.*, oxygen and carbon dioxide (*cf.* Clements, 1921; Cannon, 1925; Conway, 1940), are involved in water cultures also.

In spite of the large number of accounts written on the benefits of aeration in water cultures there is meager information in the literature concerning the concentrations of oxygen and carbon dioxide that occur in aerated and non-aerated solutions. The result has been that some investigators attribute the aerational responses of the plants to oxygen while others attribute them to carbon dioxide. Also, there is little information on the effect of oxygen tension on root structure, although data are available on the influence of various concentrations of oxygen on the absorption of ions and the production of organic acids.

This paper presents the results of an investigation of the influence of oxygen content in the nutrient solution on the growth of tomato roots. The aims were to determine the concentrations of oxygen and carbon dioxide occurring in aerated and non-aerated water cultures during a period of several weeks and to interpret the growth responses of the plants on the basis of experiments in which these factors were controlled. In a later paper the influence of oxygen concentration on root structure will be considered.

REVIEW OF LITERATURE.—The recognition of aeration as a factor in the water culture of plants was made at the time this method of growing plants was undergoing its first rapid development. Sachs (1860) recognized that a certain amount of air, in addition to the minerals, must be present in the water for the best growth of the plants.

Arker (1901) made an intensive study of the effects of aerating water, soil, and mud cultures of *Lupinus albus* and *Helianthus annuus* and found that the roots of both of these species made more rapid growth when air was bubbled through the cultures.

Hall, Brenchley, and Underwood (1914) noted a superior growth of barley and lupins in cultures of coarse sand over those in water, fine sand, or silt. While the root systems of plants in kaolin and coarse sand developed freely, those of fine sand and silt were restricted. The authors suspected aeration as the disturbing factor and tested that assumption by arranging comparative water cultures in which one series was not aerated whereas in the other bottles a continuous current of air was bubbled through the solutions. Both the barley and lupins made the greater growth in the aerated solutions, as indicated by the dry weights.

Free (1917) found that passing oxygen, nitrogen, or air through the solutions had no appreciable effect on buckwheat, when compared with open or sealed controls, but that in cultures treated with carbon dioxide the plants wilted within a few hours and died within a few days.

Stiles and Jörgensen (1917) obtained significantly better growth of barley and balsam by aerating the culture solution, but no response was obtained in buckwheat, which was in accord with the finding of Free. Stiles and Jörgensen recognized several possible factors as operating in their experiments and therefore refrained from correlating the beneficial effects of aeration with oxygen supply, removal of carbon dioxide, and other causes.

Pember (1917) did not obtain beneficial effects from daily aerations of barley.

Beals (1917) grew two *Zea Mays* plants in water culture and aerated the solution in one of the con-

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tainers. The plant in the aerated solution made the greater growth. Cross sections of the roots showed no breakdown of cells in the case of the plant from the aerated solution, but large passages were present in the cortex of the roots from the non-aerated plant.

Andrews and Beals (1919) aerated *Zea Mays* at several rates and obtained the greatest amount of growth with the greatest amount of air bubbled through the solution. In cross sections, neither aerated nor non-aerated roots were shown to have intercellular passages, although intercellular spaces in the non-aerated roots were appreciably the larger. In the latter respect the results were different from those of Beals (1917).

Andrews (1920) obtained beneficial effects from aerating *Avena sativa, Brassica alba, Pisum sativum, Fagopyrum esculentum* (buckwheat), and *Helianthus annuus*. The results for buckwheat were not in agreement with those of Free (1917) nor Stiles and Jörgensen (1917).

Allison (1921) found that the incorporation of air in the solution by the bubbling of air or by a drip solution greatly improved the growth of soybeans.

Allison and Shive (1923) grew soybeans in quart jars in simple solution, simple aerated solution, drip solution, and in aerated-drip solution. The simple solutions were renewed every three and one half days while the drip solutions received one liter of solution every twenty-four hours. In the case of the simple cultures the aeration produced no discernible benefit other than a slight increase in root growth while in the drip cultures the aeration produced a marked improvement of both top and root growth. This may be interpreted as indicating that something was more limiting than oxygen in the simple cultures and thus the beneficial effects of aeration did not become expressed under those conditions. Allison and Shive presented biweekly oxygen determination values and these showed that from the second to the eighth weeks of the experiment the oxygen content for the simple solution was 4.75, 1.25, 1.00, and 1.75 parts per million, that for the simple aerated solution was 7.00, 6.75, 7.50, and 8.00 parts per million, that for the drip solution, 6.95, 3.75, 2.95, and 3.00 parts per million, and that for the aerated-drip solution, 7.20, 6.95, 5.85, and 6.00 parts per million.

Knight (1924) obtained increased growth of *Cheiranthus* (wallflower) and *Chenopodium album* when the solution was aerated but *Zea Mays* did not respond to aeration with either a sprig of *Elodea* or the continuous bubbling of air through the solution.

Girton (1927) studied the effects of various environmental factors on citrus seedlings using water and sand cultures. Sour-orange seedlings showed significantly more root elongation in aerated than in non-aerated or pre-boiled non-aerated water cultures. Sand cultures aerated with various gas mixtures showed marked response to oxygen concentration: at 1.5 per cent oxygen no growth was made during the seventeen days of the experiment; at from 4.6 to 6.1 per cent oxygen about one half normal growth was made. In two experiments carbon dioxide was used in relatively high percentages in the gas mixtures. During seventeen days of exposure to 54.8 per cent carbon dioxide in the gas mixture the roots made only a trace of growth. Eight days of exposure to 37.4 per cent carbon dioxide in the gas mixture was accompanied by no perceptible root growth.

Loo (1928) came to the conclusion that aeration of the nutrient solution had no influence on the growth of rice, wheat, bean, and oat seedlings. However, aeration was accomplished by passing carbon dioxide-free air in a single stream of bubbles through the solution for only four minutes, three times a day.

Zimmerman (1930) studied the effect of oxygen content in water on the rooting of cuttings of several species. Willow cuttings rooted at lower oxygen levels than did more sensitive species such as tomato, salvia, and ivy, but where an oxygen gradient existed from top to bottom in the water containing stems of the former species the roots emerged from the region exposed to the greatest concentration. Aerated controls of willow, by contrast, although they showed strong polarity, produced some roots along the entire submerged portion of the stem. Tomato cuttings in deep water rotted when the containers were covered to exclude light or when the experiment was carried out in the dark. It appeared that when the green stem received sufficient light the oxygen liberated in photosynthesis was sufficient to keep the stems alive. However, root formation was erratic in cuttings in the light, unless the water was aerated.

Cannon (1932) placed sunflower plants in distilled water and kept some in the light and some in the dark. After three hours he found 10 per cent more oxygen in the water of those kept in the light than of those kept in the dark. According to Cannon (1932a) some of the oxygen liberated in photosynthesis is transported from the shoot to the root. This supplies part of the oxygen for the root and the remainder is obtained from the environment.

Clark and Shive (1932) demonstrated that for tomato continuous renewal of the nutrient solution at from one to two liters per day in two and four quart jars, depending on the size of the plants and water consumption, did not provide enough aeration even though the method of renewal incorporated a bubble of air with every drop of solution, and so the forcing of additional air through the solution improved the growth of both tops and roots. No analyses for dissolved oxygen were made but the authors assumed that this substance was the limiting factor.

Arrington and Shive (1936) showed that the aerated nutrient solution had a higher concentration of dissolved oxygen and a lower concentration

of dissolved carbon dioxide than did the non-aerated one after a six hour exposure of tomato roots.

Hoagland and Broyer (1936) concluded that oxygen must be supplied to barley roots to maintain the cell activities associated with the accumulation of salt or the retention of salt already accumulated, over a long period. Ten per cent carbon dioxide in air bubbled through the solution had no important retardation on salt accumulation and even 20 per cent carbon dioxide did not produce a very great effect under the conditions of their experiment.

Arnon (1937) obtained beneficial effects from the aeration of barley at all times of the year except during the winter.

Shive (1941) found that non-aerated soybeans depleted most of the oxygen from the nutrient solution in six to nine hours. The optimum oxygen tension for soybeans was found to be at about 6 parts per million, that for oats at about 8 parts, and that for tomatoes at 16 or more parts per million. These conclusions were based on the dry weights of the plants.

Durell (1941) aerated water cultures of tomatoes at several rates and found that aeration increased root, stem, and leaf growth and yield of fruits. The lowest rate of bubbling air through the solution was sufficient for the maximum growth of roots and production of fruits but stem and leaf growth were greatest with the highest rate of aeration.

Raalte (1941) presented evidence to show that oxygen is transported to the roots from aerial parts of rice. By this means the roots are capable of living in a medium which is nearly completely devoid of oxygen. If for any reason both internal and external supplies of oxygen are cut off from rice roots, such as when young seedlings are submerged in a poorly aerated medium, Taylor (1942) has demonstrated that the roots are able to survive by means of anaerobic respiration. The latter ability is not shared by wheat, however (Taylor, 1942).

Gilbert and Shive (1942) grew soybeans, tomatoes, and oats in solutions aerated with various partial pressures of oxygen. Soybeans showed "oxygen toxicity" at 8 and 16 parts per million of this gas in the solution. The symptoms were chlorosis and leaf distortion, occurring when a nitrate nitrogen solution was used but not when an ammonium nitrogen one was used. "Oxygen toxicity" was not found in tomato and oats.

Boynton and Compton (1943) found that a decrease in oxygen pressure of the gas continuously in equilibrium with the rooting medium to three-quarters of that found in air caused marked decreases in the number and weight of new roots and in top growth produced by apple, prune, and peach.

Pepkowitz and Shive (1944) found maximum absorption for soybeans at 16 parts per million of oxygen; maximum absorption for tomatoes was at 8 parts per million while 16 parts retarded absorption. Absorption of calcium and phosphorus was directly dependent upon the dissolved oxygen supply whereas potassium was not so materially influenced.

Vlamis and Davis (1944) found that tomato was very sensitive to oxygen content of the solution, barley only moderately so, and rice apparently not at all. However, in studying the absorption of potassium and bromide ions by excised roots it was found that the oxygen requirement in the solution was very similar for all three species. The difference in response between excised and intact roots of rice suggested the possibility of an internal supply of oxygen when the shoot was attached. The latter would substantiate the view of Cannon (1932a) and Raalte (1941). All three species were killed when carbon dioxide was passed through the solution.

Broyer (1945) found that guayule responded to aeration of the culture solution. Aeration with air containing 10 per cent carbon dioxide greatly reduced the growth, when compared with the control.

Chang and Loomis (1945) studied the effects of aeration with air, nitrogen, and carbon dioxide, as compared with unaerated controls, on absorption of various ions. Their results showed a marked variation in the degree to which absorption of the various ions was affected. Bubbling with air gave better absorption than that found in the unaerated controls, and bubbling with carbon dioxide gave a marked decrease. The authors noted that carbon dioxide treatment reduced the absorption of potassium most, and nitrogen, phosphorus, calcium, and magnesium progressively less. It might be added that air treatment increased absorption of potassium most and nitrogen, phosphorus, calcium, and magnesium progressively less.

Leonard and Pinckard (1946) found that the apparent optimum concentration of oxygen for cotton-seedling root elongation in water culture was between 7.5 and 21 per cent when the carbon dioxide was held constant at 10 per cent. Aerating with "zero" oxygen in the gas mixture did not kill the roots but prevented their growth. At oxygen concentrations of 90 and 100 per cent both root and top growth were restricted. While tap root elongation increased up to 15 per cent oxygen, the weight of the root system was near or at its maximum at 2.5 per cent oxygen. The lack of increase in weight of the roots with increased rate of growth was attributed to the greater thickness of roots produced at the lower oxygen levels. Concentrations of carbon dioxide up to 15 per cent seemed to have no deleterious effects on the root growth of cotton. At 30 and 45 per cent, however, root growth was retarded. Sixty per cent or more carbon dioxide prevented growth.

MATERIALS AND METHODS.—Tomato plants (*Lycopersicum esculentum,* var. Marglobe) were started in sand and transferred to nutrient solution as soon as the true leaves began to appear between the cotyledons (about ten days). The composition of the solution was that given by Hoagland and Arnon (1938), with all nitrogen in the nitrate form and

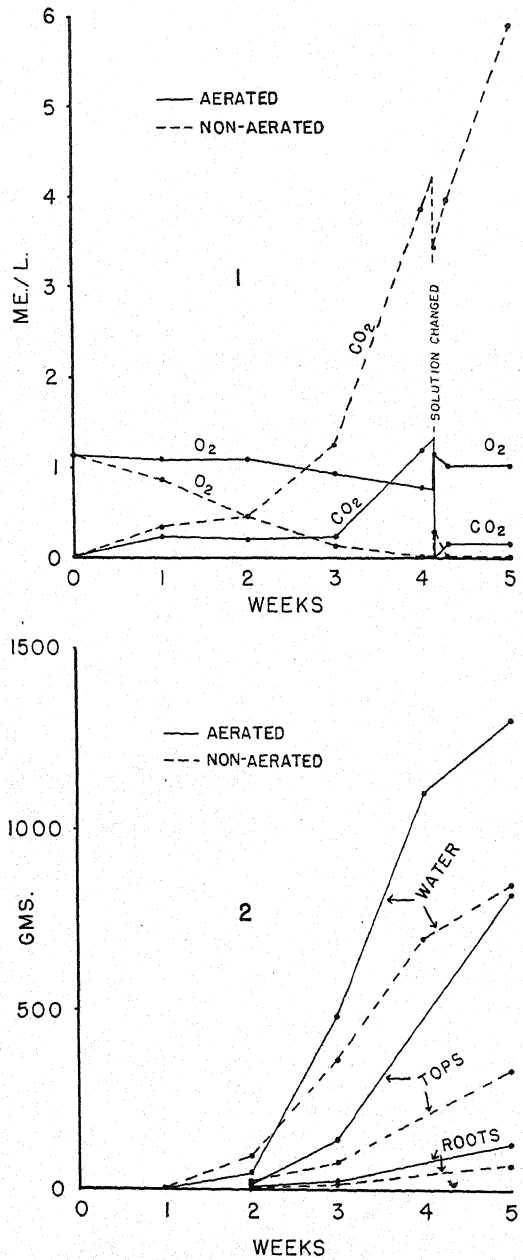


Fig. 1–2.—Fig. 1. Mean oxygen and carbon dioxide (undissociated) contents (milliequivalents per liter) found in aerated and non-aerated water cultures of tomatoes during a five-week period. Four plants to three gallons of solution replicated four times.—Fig. 2. Water consumption per day and fresh weights of tops and roots of tomato plants growing in aerated and non-aerated water cultures during a five-week period. Means of 4 four-plant replicates.

iron as ferric sulfate. Three types of experiments were conducted: the first to demonstrate the effects of aeration on tomato and to determine the extent to which oxygen was depleted and carbon dioxide accumulated in non-aerated culture; the second to determine the effects on root growth of various concentrations of oxygen; the third to determine the degree of toxicity of certain concentrations of carbon dioxide. All experiments were carried out in the greenhouse from May through September, 1945, at Salinas, California.

Oxygen determinations were made by Winkler's method, as outlined by the Association of Official Agricultural Chemists (1940).

Carbon dioxide determinations were made by a method modified from that outlined by the Association of Official Agricultural Chemists (1940) for carbonate determinations of soil. Essentially, the method consisted of bubbling carbon dioxide-free air through an acidified sample and then recovering in an absorption tower containing a known amount of sodium hydroxide solution the carbon dioxide liberated by the sample. The base plus dissolved carbon dioxide in the absorption tower were transferred quantitatively to a titration flask and an excess of barium chloride added, after which hydrochloric acid was titrated against the remaining sodium hydroxide, using phenolphthalein as an indicator.

Collection of the samples of nutrient solution for carbon dioxide determinations was made in a 100 ml. pipette marked at 1 and 101 ml. One milliliter of sodium hydroxide solution was sucked into the tip of the pipette with the aid of a siphon bottle and then the pipette was dipped into the culture solution and 100 ml. of sample sucked in. The purpose of the base was to raise the pH of the sample and convert the carbon dioxide to the carbonate form, preventing loss of gas to the air. The bulk of the sample was expelled from the basal end of the pipette into a test tube of 125 ml. capacity, but the last few drops were delivered in the normal manner to obtain the standard amount from the pipette. This method of emptying the pipette served two purposes: to mix the last-taken few milliliters of sample with the base in the bulb of the pipette before exposure to the air, and to decrease the time of exposure of the sample to air during delivery.

A correction was made for the small increase of carbon dioxide derived from the air during various steps in the procedure. This was done by analyzing samples of boiled nutrient solution to obtain a zero value. The results of the analyses are presented as milliequivalents of undissociated carbonic acid per liter of nutrient solution. The values were calculated from the total carbon dioxide, the pH of the solution, and the primary dissociation constant of carbonic acid.

Water consumption by the plants was determined for twenty-four-hour periods in the first experiment by observing the change in water level in large graduated cylinders inverted over small dishes which were joined by means of siphons to the jars containing plants. Water lost through evaporation was estimated by measuring loss from jars without plants.

Treatments of plants, in all experiments, were

replicated four times, and single analyses were made on all four replicates.

EXPERIMENTAL.—*Aerated and non-aerated cultures.*—In this experiment the plants were grown for a maximum time of five weeks in three-gallon glazed jars with four plants per jar. The solution in one half of the jars was aerated with compressed air bubbled from two-inch pieces of porous carbon tubing of the type described by Arnon and Hoagland (1940), while the solution of the others had only such gas exchange as occurred by diffusion and with the addition of water and iron. The temperature of the solution was uncontrolled but it varied somewhat less than did that of the air of the greenhouse, which ranged from 60 to 80°F., daily. Weekly analyses for dissolved oxygen and carbon dioxide were made on four replicates; water consumption for a twenty-four-hour period was also measured weekly. Harvests were made at the end of two, three, and five weeks for determination of weights. Iron was added semi-weekly; on the twenty-first day additional microelements were added; and on the twenty-ninth day the nutrient solution was changed. At the latter time the diphenylamine test indicated that the nitrates were practically exhausted from the aerated solution but were still relatively abundant in the non-aerated one.

The new solution, as put into the aerated jars, was in equilibrium with the air with regard to dissolved oxygen and carbon dioxide, but the new solution added to the non-aerated jars contained these gases in approximately the same concentrations that they occurred in the old solution. This was effected by bubbling nitrogen through the bulk of the new solution for a few hours to drive off the oxygen, and then by adding enough water saturated with carbon dioxide to produce the desired final concentration of minerals and carbon dioxide. However, loss of carbon dioxide and gain of oxygen during the handling of the solutions caused slight deviations (fig. 1).

An attempt was made to maintain the solution between pH 5.0 and 5.6 but near the end of the experiment the hydrogen ion concentration decreased so rapidly in the aerated solution that the pH rose to 6.0 or slightly higher for short periods between daily adjustments.

The results of the experiment are presented in figures 1–6. In the aerated solution the oxygen content remained high, or practically saturated for the prevailing temperatures, until the third week. At this time and at the end of four weeks it was observed that the oxygen content was beginning to decrease considerably. This period coincided with a rapid increase in the size of the plants, especially in the mass of roots. At the time the solution was changed on the twenty-ninth day new aerators were installed so that a greater volume of air would be delivered through the solution to maintain a more nearly saturated condition. Subsequent analyses of the solution showed that the oxygen content remained high.

In the non-aerated solution, on the other hand, the oxygen content showed a noticeable decrease in concentration even during the first week when the tomato plants were quite small. At the end of two weeks there was less than one half as much as in the aerated solution. After three weeks and longer the value decreased to less than one tenth as much oxygen as in the aerated cultures. Only for a few hours after the solution was changed on the twenty-ninth day was the oxygen content in the non-aerated solution any greater.

The carbon dioxide concentration found in the freshly prepared nutrient solution was below the sensitivity of the method used for analysis. After one week, however, a detectable amount was found in the aerated solution. At the end of four weeks the concentration was slightly over one milliequivalent per liter. This corresponded in time with the low value for oxygen content in the aerated solution and was undoubtedly the result of clogging aerators and thus insufficient aeration to flush the carbon dioxide from the solution. After changing the solution and aerators, the carbon dioxide remained at about its early level.

The carbon dioxide content of the non-aerated solution rose slowly at first but at the end of three weeks and after, it rose very rapidly and was still rising at the end of the experiment after five weeks. It attained, in this time, a value of a little under 6 milliequivalents per liter. When the solution was changed on the twenty-ninth day the new solution had slightly less carbon dioxide than the old so the accumulation was set back somewhat more than twenty-four hours.

The water consumption was not accurately measurable until after the second week. This was mainly due to the fact that evaporation was much greater than the amount of water used by the plants. The amount of water used per day at the end of five weeks by the plants in the aerated solution would probably have been greater than it was (fig. 2) if adequate minerals had been available. Even though the solution was changed a few days before, the nitrates were about depleted, and were probably the limiting factor at the time the experiment ended. However, the water consumption was one and one half times as much as for the non-aerated plants.

The non-aerated plants showed temporary wilting for several days beginning with the sixteenth, indicating that insufficient water was being absorbed in the limiting solution.

Growth of the plants was about equal for the first two weeks for both aerated and non-aerated conditions. After this time the non-aerated plants became increasingly limited by lack of aeration of the solution. At the end of three weeks the non-aerated were slightly more than one half the weight of the aerated ones and a difference in size was evident. Leaves and stems were smaller and a difference in the habit of the roots was developing. The roots of the non-aerated plants were shorter and many new ones were developing near or above

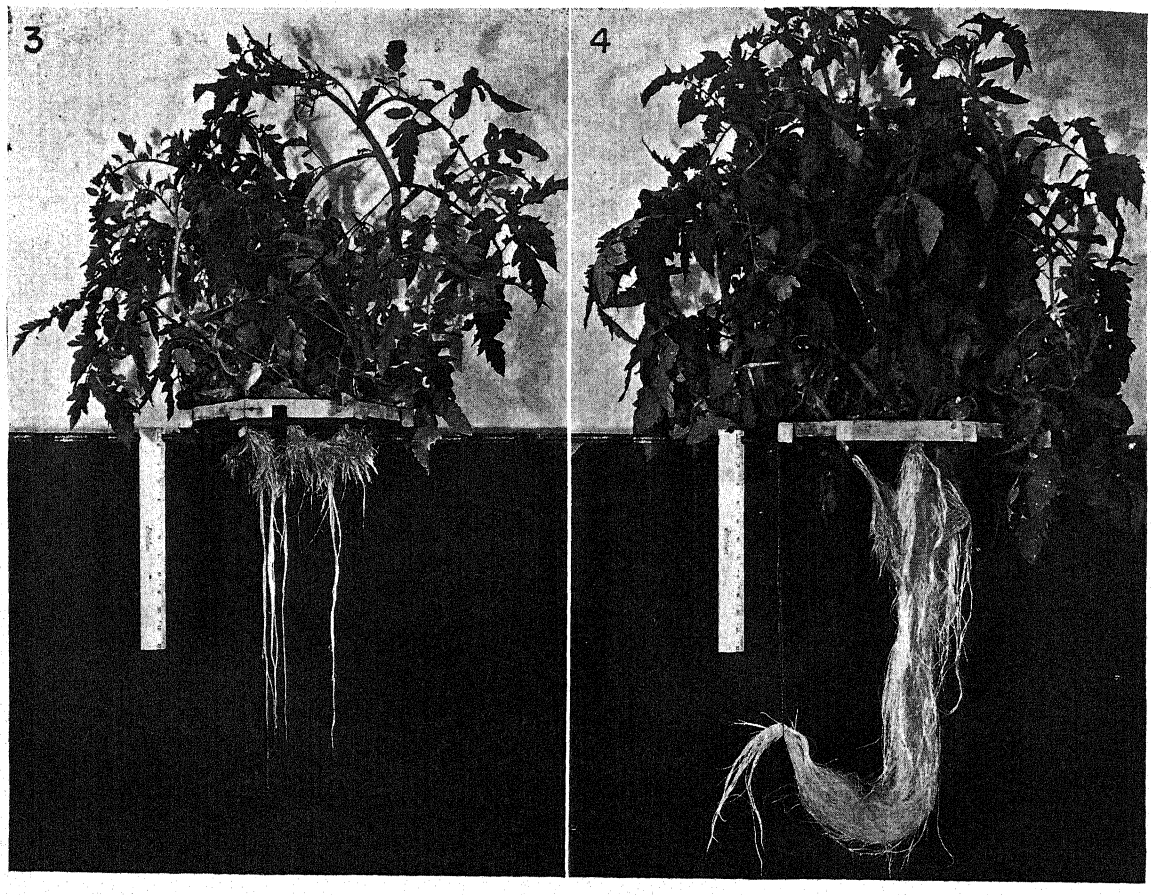


Fig. 3–4. Tomato plants after five weeks in non-aerated and aerated water cultures; four plants in three gallons of solution.—Fig. 3. Non-aerated; O_2 = 0.041 me./l. (0.71 per cent); CO_2 = 5.9 me./l. (6.8 per cent). The longer roots, produced at a time when the oxygen content was higher, were dead.—Fig. 4. Aerated; O_2 = 1.07 me./l. (18.3 per cent); CO_2 = 0.15 me./l. (.2 per cent).

the surface of the solution. The uppermost roots tended to grow horizontally in some cases and a few were even turned up at the tips.

From the third to the fifth weeks the non-aerated plants showed increasing limitation of both top and root growth (fig. 3–4). Although stems grew nearly as lengthy as in the case of the aerated plants, they branched less and were not as thick. Leaves were smaller and somewhat darker green than those of the aerated plants, but the leaves of the latter could not be called chlorotic.

During the early part of the experiment the roots of non-aerated plants extended far into the solution, but before the termination of the experiment these roots were all dead and only some of those near the surface remained alive (fig. 3). Probably the diffusion of oxygen from the air above the solution and constant replacement of absorbed and evaporated water supplied enough oxygen to keep some alive. The matted roots were partly in the solution and partly above. The habits of individual lateral roots are shown in figure 5. Under both conditions they became densely covered with slender hair roots.

The results in this experiment were similar to those reported in instances previously cited, and while those investigators concluded that oxygen was perhaps the limiting factor in non-aerated cultures, none determined the changes in oxygen and carbon dioxide contents of the solutions over a long period of time. To determine the extent to which each, oxygen and carbon dioxide, were limiting in the non-aerated cultures of tomato, experiments were designed to vary one gas concentration at a time.

Aeration with gas mixtures containing various amounts of oxygen.—This experiment was designed to test the effects of various concentrations of oxygen in the nutrient solution when carbon dioxide was constant. Primary interest lay in the lower concentrations of oxygen but it seemed desirable to test concentrations higher than that obtained with air. The latter was done separately because of limitations of equipment and the number of analyses required in a short time.

For the low range of concentrations of oxygen, plants were grown in twenty two-quart jars, three

TABLE 1. *Characteristics of tomato plants after two weeks in water culture aerated with mixtures of oxygen and nitrogen; three plants in two quarts of solution, constant temperature of solution 25°C., mean values for four replicates.*

	Dissolved O_2, me./l.					Amt. req. for signif.[b]	
	.049±.006[a] (0.98%)	.150±.012 (3.03%)	.256±.011 (5.20%)	.492±.004 (9.92%)	.989±.009 (19.9%)	5%	1%
Mean maximum root length, cm...	7	18	30	37	49	3.2	4.4
Mean stem length, cm.....	10.9	13.3	13.5	14.6	13.7	1.1	1.4
Mean dry weight of tops, g.....	1.31	2.44	2.68	2.78	3.11	.20	.28
Mean dry weight of roots, g.....	.23	.53	.70	.74	.78	.07	.10
Mean top-root ratio (dry weight)	5.88	4.47	3.86	3.77	4.05	.38	.52

[a] Standard error. [b] Hayes and Immer, 1942.

plants per jar, in temperature tanks[2] (Campbell and Presley, 1945) controlled at 25°C., for a period of two weeks; and were aerated during that time with mixtures of oxygen and nitrogen which gave the following approximate concentrations of dissolved oxygen in the solutions: 0.05, 0.15, 0.25, 0.50, and 1.0 milliequivalents per liter. The lowest concentration was obtained with unpurified cylinder nitrogen, the highest with compressed air, and the three intermediate concentrations by mixing oxygen and nitrogen. The exact analytical values are presented in table 1. The initial pH of the solution was 5.6. On the twelfth day the solution was renewed but only after the new solution had first been aerated with the appropriate gas mixture for approximately three hours.

Representative plants are shown in figure 7 and a summary of the data with statistical analyses (Snedecor, 1940; Hayes and Immer, 1942) is presented in table 1. The lengths of the roots showed a decided response to the concentration of oxygen in the solution. The tops also showed a significant trend toward increasing weight with higher concentration of oxygen. The top to root ratio, based on dry weights, also showed significant differences between treatments. In other words, the smaller root systems supported disproportionally larger tops.

The highest concentration of oxygen used in the previous experiment was obtained with air, but there was no indication that the optimum concentration for root growth had been reached. Vlamis and Davis (1944) obtained as much absorption of potassium and bromide in excised tomato roots at 9.5 per cent oxygen as at 21 per cent, but a slightly higher value was obtained with both ions at 100 per cent oxygen. Leonard and Pinckard (1946) found that optimum aeration of cotton was reached with air and it was only at 90 or 100 per cent oxygen that root growth was retarded.

To test the effects of higher concentrations of oxygen in the solution on the growth of tomato roots, plants were started in the temperature tanks as before and allowed to grow for two weeks. The root systems were then cut to three inches and new vigorous roots allowed to grow out. On the third day following the cutting, the solution was changed and on the fourth day the aeration with mixed gases was begun.

After three hours of aerating the solution, the roots were marked with a precipitate of iron tannate made as follows: the roots were dipped for about thirty seconds in a 0.2 per cent solution of tannic acid (in tap water, which gave pH 6.4), then for about 5 seconds in a 0.1 per cent solution of ferric sulfate. This was repeated once with a shorter period of time in the tannic acid, and was followed by rinsing in tap water.

Analyses of the solutions were made on the next day, prior to terminating the experiment, and the values obtained for dissolved oxygen in milliequivalents per liter were: 1.0, 1.8, 2.7, 3.5, and 4.6 (approximately 20, 37, 55, 75, and 95 per cent oxygen, respectively).

TABLE 2. *Amount of growth made by tomato roots during twenty-four hours in water culture at 25°C., aerated with several concentrations of carbon dioxide (oxygen constant at about 1 milliequivalent per liter, ca. 20 per cent; means of 100 roots each).*

Undissociated H_2CO_3		Initial pH, 4.5	Root growth
me./l.	% sat.	Final pH	mm.
0.065	0.1	5.1	29.1
1.54	2.3	4.8	31.8
3.28	5.1	4.9	31.2
5.96	9.1	4.7	24.4
18.9	28.8	4.6	17.1
Amt. req. for signif. at 5%			7.2
Amt. req. for signif. at 1%			10.0

At the end of twenty-four hours' growth in the solutions aerated with the gas mixtures, the twenty-five roots showing the most new growth in each of four replicates were cut off and kept in petri dishes containing water to which a few drops of formalin had been added, until measured.

The roots are shown in figure 8. There was no significant difference in any of the groups except that which had been aerated with commercial oxy-

[2] The author is grateful to Dr. W. A. Campbell of the Special Guayule Research Project for extending the use of the temperature tanks at Salinas.

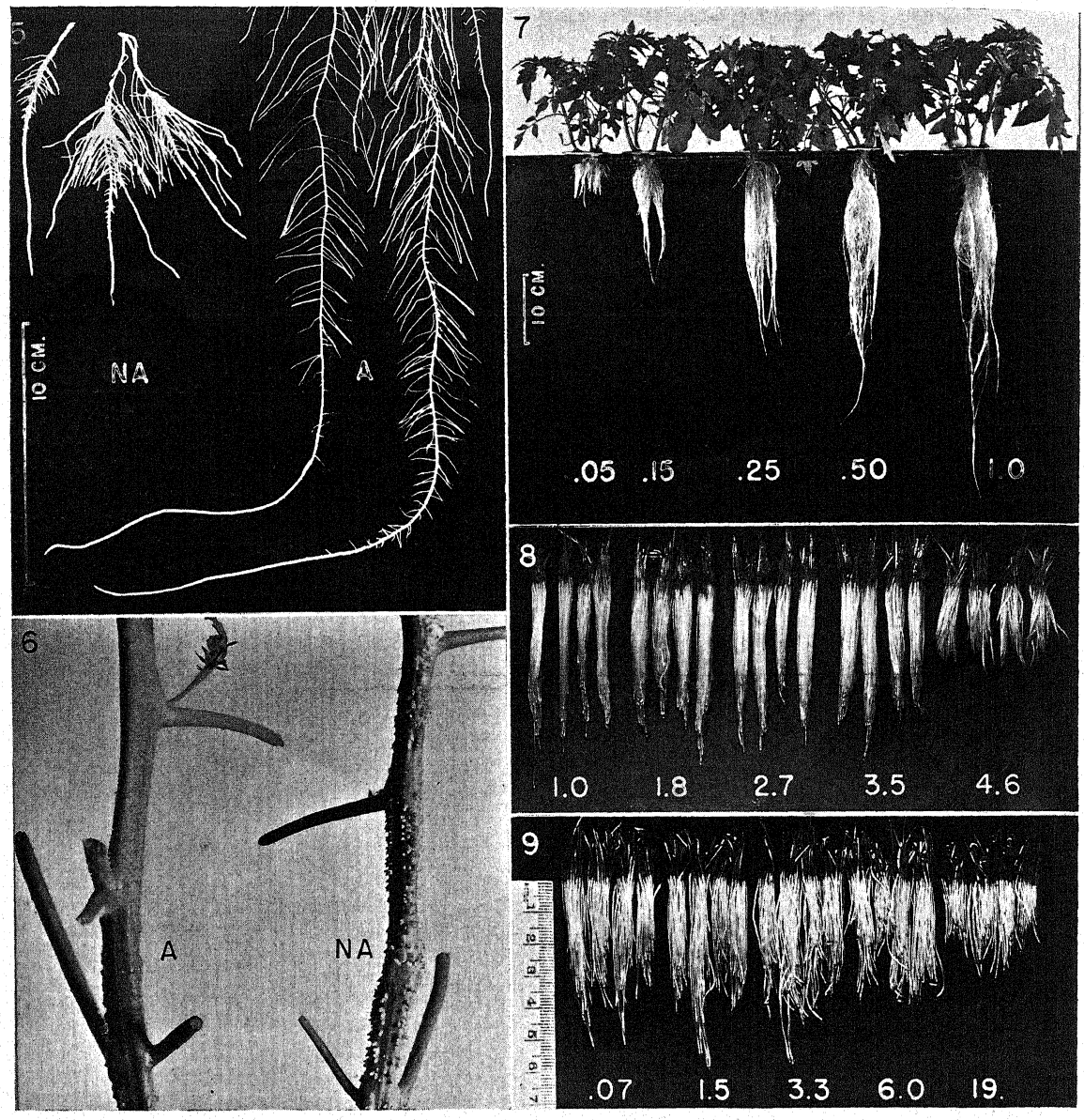


Fig. 5–9.—Fig. 5. Roots from tomato plants after five weeks in non-aerated (NA) and aerated (A) water culture. The aerated roots correspond to roughly the lower third (in length) of the others. The central root tip of the non-aerated roots was dead.—Fig. 6. Stems of tomato plants from aerated (A) and non-aerated (NA) water cultures showing the striking difference in the numbers of adventitious roots forming. The lowest petiole stubs in each case are those of the first leaves above the cotyledons.—Fig. 7. Tomato plants after two weeks in water culture aerated with mixtures of oxygen and nitrogen; three plants in two quarts of solution, constant temperature of solution, 25°C. Oxygen concentrations in the solutions were 0.05, 0.15, 0.25, 0.50, and 1.0 milliequivalents per liter (approx. 1, 3, 5, 10, and 20 per cent of saturation, respectively, at that temp.).—Fig. 8. Growth of tomato roots made during 24 hours in water culture at 25°C. aerated with mixtures of oxygen and nitrogen, and containing 1.0 1.8, 2.7, 3.5, and 4.6 milliequivalents of oxygen per liter of solution (approx. 20, 37, 55, 75, and 95 per cent of saturation, respectively, at that temp.). In each replicate the twenty-five roots with most growth were chosen.—Fig. 9. Growth of tomato roots made during 24 hours in water culture at 25°C. aerated with mixtures of oxygen (constant at about one me./l.), nitrogen, and carbon dioxide, and containing 0.07, 1.5, 3.3, 6.0, and 19 milliequivalents of undissociated carbonic acid per liter (approx. 0.1, 2.3, 5.1, 9.1, and 28.8 per cent of saturation, respectively, at that temp.). In each replicate the twenty-five roots with most growth were chosen. Statistical treatment presented in table 2.

gen, and here the amount of growth was significantly less than for the others. McPherson (1939) stated that excessive oxygenation of *Zea Mays* roots retarded their growth. Thus *Zea Mays,* cotton

(Leonard and Pinckard, 1946), and tomato show a uniform response to high concentrations of oxygen in the solution.

Aeration with gas mixtures containing various amounts of carbon dioxide.—Oxygen, nitrogen, and carbon dioxide were mixed in cylinders by partial pressures. In each case the partial pressure of oxygen was made one fifth of the total pressure (about 1.0 milliequivalent per liter). The addition of various amounts of carbon dioxide displaced nitrogen from the basic mixture of air. The carbon dioxide concentrations found in the solutions were 0.065, 1.54, 3.28, 5.96, and 18.9 milliequivalents per liter (about 0.1, 2.3, 5.1, 9.1, and 28.8 per cent, respectively).

Plants were grown in the two-quart jars in the temperature tanks as in the previous experiment and the only differences were that the nutrient solution was changed on the day of starting the special aeration, and the new solution was made more acid (pH 4.5) to prevent appreciable dissociation of the carbonic acid.

The roots are shown in figure 9 and the statistical treatment is presented in table 2. Although a highly significant reduction of growth occurred with the highest concentration of carbon dioxide, this treatment also had a significantly higher concentration of hydrogen ions at the end of twenty-four hours (table 2). In view of the results of Arnon and Johnson (1942) a slight difference in hydrogen ion concentration in this range (pH 4 to pH 5) is critical for tomato. Thus, the toxicity of carbon dioxide in this experiment was confounded to some extent with the toxicity of hydrogen ions as such. In any event the total toxicity of the solution was insufficient to reduce growth by quite 50 per cent of the controls. Leonard and Pinckard (1946) found that at 30 per cent carbon dioxide cotton roots also show a definite retardation of root growth.

Discussion.—The results obtained with tomato plants grown in aerated and non-aerated cultures gives additional evidence that aeration is important for obtaining the maximum growth of this species in water culture. The results are in agreement with those of Clark and Shive (1932), Arrington and Shive (1936), Durell (1941), and Vlamis and Davis (1944). On the other hand, Went (1943) obtained more rapid stem growth with tomato plants in unaerated solution than in aerated when he induced additional roots to develop out of the nutrient solution. The hypothesis of the latter author is that a factor for stem growth, "caulocaline," is produced by the roots, and in the case of tomato some of the roots seemed to need to be in contact with moist air to supply enough of this substance for maximum stem growth. Went concluded that when roots had developed in a moist, inert, porous medium above the nutrient solution, aeration of the nutrient solution was of no importance for growth of the tomato plants. In some experiments he found that aeration decreased the growth rate in direct proportion to the amount of air passed through the solution. However, when the high rates of aeration were used, the physical disturbances to the roots may have had important consequences, for in the present investigation equal rates of bubbling were used and differences in oxygen content achieved by variation of the partial pressure of this gas in the mixture. Under these conditions the oxygen content obtained by bubbling air through the solution resulted in better growth than did a lower oxygen content; and a wide range of oxygen contents above that produced by air had no ill effect on the rate of root growth.

The lowest value for oxygen that Allison and Shive (1923) found in non-aerated cultures of soybeans (1.0 p.p.m. or about 0.125 me./l.) was slightly more than twice the value found in the present study on tomato (0.05 me./l.). The significance of these quantities and the difference between them will become evident only after a considerable number of species have been studied similarly. Arrington and Shive (1936) determined changes in both oxygen and carbon dioxide contents of aerated and non-aerated solutions of tomato but the experiment was continued only long enough to show significant differences (six hours).

It was demonstrated in the present investigation that aeration of a proper sort maintained a high concentration of oxygen in the solution and at the same time flushed out the carbon dioxide liberated from the roots. The very similar growth produced by the tomato plants in aerated and non-aerated solution while they were small indicates that the necessity of aeration arises only because of the inadequate rate of transfer of these gases by diffusion. Under conditions of low oxygen consumption by the plants and features promoting a maximum rate of oxygen diffusion into the solution it might be expected that differences in growth between aerated and non-aerated cultures would be difficult to demonstrate. This brings up a consideration which is very frequently obscured in the literature, namely, that aeration is a means whereby such factors as oxygen and carbon dioxide are regulated and is not a simple factor itself. Obviously, if aeration is instigated to supplement the oxygen content of the solution no response would be obtained from this factor if the solution were already well supplied, even though the plant had a high requirement for this gas. Thus, if growth were limited by other factors, the need for aeration would not be as great. In this regard, Arnon (1937) found that barley responded to aeration at every season except winter. Clark and Shive (1932) found that the differences in weights between aeration treatments of tomatoes varied with the season and duration of the experiment.

Evaluation of the concentrations of oxygen and carbon dioxide found in the solutions of the first experiment were made possible by the experiments in which these gases were varied individually. From the results of the latter it was indicated that beginning with the third week and continuing through-

out the remainder of the experiment the oxygen content of the non-aerated solution was limiting root growth, at first slightly and later severely. The carbon dioxide content of the non-aerated solution, on the other hand, rose to what appeared to be serious proportions, when compared with the magnitude of the oxygen changes, but judging by the results of the carbon dioxide experiment, it reached a value which might have been slightly limiting only at the close of the experiment.

Andrews and Beals (1919) and Durell (1941) made rough quantitative determinations of the amount of aeration needed for the best growth of the species they studied, but the lack of any solution analyses left the critical conditions of the solutions unknown. To obtain a fundamental basis for the rate of aeration it is necessary, first of all, to determine the optimum concentration of oxygen in the solution for the species under consideration. Gilbert and Shive (1942) came to the conclusion that species may vary greatly in their oxygen tension requirement; *e.g.*, tomato has a high requirement, and an example of a plant with a very low oxygen requirement in the root substrate is rice (Vlamis and Davis, 1944). With such information and a solution analysis it is possible to estimate the degree to which a certain rate of aeration is limiting.

Vlamis and Davis (1944) found that 2.7 per cent oxygen (about 0.13 me./l.) was almost enough for maximum absorption of potassium and bromide ions by excised roots when time was not a factor. However, relative oxygen consumption by excised roots showed an increase up to 21 per cent oxygen. The latter parallels closely the increased growth rate of roots found in this study for oxygen concentrations up to 1.0 milliequivalents per liter (about 20 per cent). Pepkowitz, Gilbert, and Shive (1944) found that an oxygen concentration of 4 parts per million in nutrient solution (about 0.5 me./l. or 10 per cent) resulted in anaerobic or semi-anaerobic respiration in oat roots, with the production of large amounts of organic acids, as compared with roots exposed to 8 or 16 p.p.m. of oxygen. Ulrich (1942), on the other hand, failed to influence the organic acid content of excised barley roots by changes in oxygen tension between 3.2 and 88 per cent. This was explained on the basis that the function of the acids was in maintaining the acid-base balance of the cell sap. Since the variation of oxygen tension under the conditions of the experiment did not affect the hydrogen ion concentration there was no change in organic acid content.

SUMMARY

An investigation was made of the influence of oxygen content in the nutrient solution on the growth of tomato roots. Three types of experiments were conducted: (1) Plants were grown in aerated and non-aerated culture solutions for five weeks, and oxygen and carbon dioxide determinations were made at weekly intervals. (2) Plants were grown in water cultures maintained at 25°C. and aerated with mixtures of gases in which the partial pressure of oxygen was varied. (3) Plants were grown in water culture maintained at 25°C. and aerated with mixtures of gases in which the partial pressure of carbon dioxide was varied.

Plants in non-aerated solution showed increasing limitation of growth and water consumption from the second to the fifth weeks, when compared with the plants of the aerated solution. In the aerated solution the oxygen and carbon dioxide contents remained near their initial high and low values, respectively, while in the non-aerated solution the oxygen content decreased to 0.05 milliequivalents per liter (about 1 per cent) and the carbon dioxide accumulated to 5.9 milliequivalents per liter (about 6.8 per cent) during the five weeks of the experiment.

Aerating with gas mixtures containing less oxygen than is present in the air resulted in an inferior growth rate of both roots and tops, when compared with that of the controls. Aerating with gas mixtures containing more oxygen than is present in the air resulted in no difference in the rate of root growth except at the highest value (obtained with commercial oxygen) and in this case the growth was significantly retarded.

Aerating with gas mixtures containing various amounts of carbon dioxide up to 18.9 milliequivalents per liter (about 28.8 per cent) showed that at the latter value the growth rate of roots was limited by a highly significant amount, part of which limitation may have been confounded by changes in hydrogen ion concentration. Roots of the plants in the solution with 5.9 milliequivalents of carbon dioxide per liter (about 9.1 per cent) were barely significantly shorter (5 per cent level of probability) than the controls.

Interpretations of the growth responses obtained in the aerated and non-aerated solutions with data obtained from the experiments in which constant levels of oxygen and carbon dioxide were maintained indicated that the characteristic differences observed between tomatoes in aerated and non-aerated water cultures were produced by insufficient oxygen in the solution and that these differences developed before the concentration of carbon dioxide reached a value that might have been slightly toxic.

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SEED DEVELOPMENT IN ACALYPHA RHOMBOIDEA AND SOME OTHER EUPHORBIACEAE [1]

Margaret Landes [2]

In the course of a study on *Acalypha rhomboidea* Raf. to determine the chromosome number, a few ovules in late stages of development were examined. It was noted that vascular strands were present in tissue that apparently belonged to the nucellus. Since this has been seldom reported, it was thought worth while to make a more complete investigation on this species and some other members of the Euphorbiaceae.

The earlier cytological literature dealing with Euphorbiaceae has been summarized by Schürhoff (1924); descriptions of their embryogeny have been brought together by Schnarf (1929), and those of seed anatomy by Netolitzky (1926). These reviews do not include a paper by Mandl (1926) which reports that short branches of the vascular bundle lie in the nucellus of the species of *Euphorbia* that he studied, one by Wiehr (1930) on the histology of mature seeds of a number of species belonging to 7 genera of the Euphorbiaceae, and one by Maheshwari and Johri (1940) on *Acalypha indica* which deals mainly with the origin of the nucellar beak and deviations that occur in the development of the embryo sac.

Materials and methods.—Staminate and pistillate flowers of *Acalypha rhomboidea* Raf. were collected in 1940 along Madison streets and, in 1941, from the university campus near the lake shore where the species grows luxuriantly. Belling's and Randolph's modifications of Navashin's fixative were used chiefly; a small amount of material was fixed in Juel's solution. Sections were cut from 10 to 15 μ in thickness. The stains used included fast green and safranin, Flemming's triple stain, Conant's quadruple stain, Heidenhain's and Delafield's haematoxylin, and Newton's crystal violet-iodine combination. The last proved most satisfactory.

Flowers of other Euphorbiaceae, mostly from Wisconsin, were similarly fixed, sectioned and stained for comparison with *Acalypha rhomboidea*. These species include *Croton glandulosus* var. *septentrionalis* Muell. Arg., collected at Lodi; *Euphorbia dentata* Michx. and *E. nutans* Lag. from Monona Bay; *E. maculata* L. from the university campus; *E. Esula* L. and *E. corollata* L. from Middleton; *E. marginata* Pursh from Atchison, Kansas, and also from near Lodi, where it had escaped from a garden; and *Codiaeum variegatum* Blume from a single plant in the botanical greenhouse.

Representative plants of each of these species except *Euphorbia marginata* Pursh and *Codiaeum variegatum* Blume have been placed in the university herbarium.

Observations.—*A. rhomboidea.*—In *Acalypha rhomboidea,* as in most Euphorbiaceae, the ovary is trilocular. A single ovule arises from the placenta at the base of each locule (fig. 1). However, more rapid growth of the basal part of the placenta and ovule soon carries the attachment region upward. The vascular supply to the ovary gives off a trace to each carpel. Each carpel supply branches again into a dorsal trace to the wall of the carpel and a ventral trace to the placenta and ovule. At first the ventral trace is "S-" shaped (fig. 2); but, as growth progresses, it takes a sharp turn from the placenta into the funiculus so that the courses of its upward and downward parts are approximately parallel (fig. 5). The mature ovule is anatropous.

A hypodermal archesporial cell is differentiated while the ovule is attached near the base of the locule (fig. 1). That this cell divides to form a primary wall cell and the macrospore mother cell is inferred from the fact that at the first stage at which the latter has been seen, it is covered by at least three layers of nucellar cells. The attachment of the ovule is now toward the upper part of the locule. In the meantime the obturator and the integuments make their appearance (fig. 2). Through division of the cells in the micropylar end of the nucellus, a beak five or more cells in length is developed which is in contact with the obturator. The obturator, arising from the placenta above the ovule, fits like a hood about the nucellar beak. It extends downward, at least on the placental side, as far as the distal end of the inner integument. The inner integument covers approximately the chalazal third of the nucellus. However, the outer integument has grown upward over most of the nucellus and part of the obturator (fig. 3). Each integument is three cell-layers in thickness.

The macrospore mother cell, which functions directly in the formation of the embryo sac, enlarges considerably before the first division occurs. Twenty-one pairs of chromosomes were observed at diakinesis (fig. 4). No cell division takes place after either meiotic division, but a thickening of the spindle fibers in the equatorial region was noted following the first division (fig. 6). Of the four nuclei resulting from the second meiotic division (fig. 7), one comes to lie at each end and one on each of two opposite sides of the sac (fig. 8). Spindle fibers are present connecting the four nuclei, but no cell-plate formation has been seen. Later, the fibers disappear and a large vacuole occupies the center of the cell. Two nuclear divisions follow, so that sixteen nuclei are present in the embryo sac (fig. 9, 10). In the mature sac, be-

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[2] This work was done at the University of Wisconsin in partial fulfilment of the requirements for the degree of Doctor of Philosophy. The author wishes to express her appreciation to Dr. C. E. Allen for his kind supervision and helpful criticism.

sides the egg apparatus and the three antipodal cells, there are two lateral groups of three cells each; four nuclei, forming a central group (fig. 11), remain distinct until after fertilization. Fertilization has not been observed with certainty but, as nearly as can be determined, the expected 5 *n* number of chromosomes (105) appears in the divisions of endosperm nuclei (fig. 12). (Forty-two chromosomes were counted in cells of the root tips (fig. 13).)

After fertilization has taken place, the obturator ceases to grow. At the two-celled proembryo stage it appears in contact only with the very tip of the nucellar beak (fig. 14). Eventually it lies entirely outside the integuments (fig. 18). Most of the outer integument remains three cell layers in thickness, but at the micropylar end it is relatively massive. This part becomes the caruncle of the mature seed. Striations mark the walls of the inner epidermis of the outer integument. The inner integument finally covers the nucellar beak (fig. 14) and shortly afterward closes over it (fig. 16). Its outer and inner layers become conspicuously differentiated (fig. 15, 17). As reported by Netolitzky (1926) for many other members of the Euphorbiaceae, the outer epidermis later becomes the brittle stony layer of the seed coat. The cells of the inner epidermis of the inner integument become filled with coarsely granular material and the walls become thickened. As growth of the ovule continues, the granular material disappears; the cells become flattened (fig. 20, 21); band-like thickenings appear on the tangential walls (fig. 22). Since the inner epidermis of the inner integument is always so sharply defined, the nucellus can be readily identified at all stages of development. Within the nucellus, a central strand consisting of a column of cells more richly protoplasmic and more regular in arrangement than those of the surrounding tissue, is differentiated from above the chalaza to the antipodal end of the embryo sac (fig. 21). There is no connection between it and the vascular supply. It can be recognized in young ovules when a proembryo is present. What seems to be a comparable structure is called a "hypostase-like" strand by Wunderlich (1938) and Sundar Rao (1940).

Except for the four conspicuous suspensor cells, the embryo is spherical from an early stage until the cotyledons are differentiated (fig. 16). The embryo sac becomes filled with cellular endosperm. Cells with thickened walls in the region above the vascular strand become stored with granular material which later disappears. This region will be referred to as the *hypostase*. A chalazal pocket is differentiated at the upper end of the central strand. The cells of the nucellus immediately surrounding the central strand become large and vacuolate; those adjacent to the inner integument (fig. 17) and the cells of the nucellar beak retain their protoplasm. Vascular elements are differentiated in the chalaza as far as the region just below the hypostase. Except for an increase in size there is little difference in the appearance of the ovule until well after the cotyledons are differentiated (fig. 19).

Before the ovule is fully mature, a well-differentiated embryo is present. The epicotyl lies between wide, flat cotyledons. Vascular tissue is well-defined in the hypocotyl (fig. 20), and it branches freely in the cotyledons. The endosperm is filled with uniformly distributed food material, except that at the chalazal end there remains a single layer of cells in which storage products are lacking and which lies in close contact with the persistent nucellus. At an earlier stage, this layer of cells was densely protoplasmic, in marked contrast to the vacuolate cells of the remainder of the endosperm. Between the endosperm and the nucellus at the chalazal end is a layer of material which is either a secreted substance or a modification of the cell walls of the endosperm and nucellus. This is most marked in the region of the chalazal pocket. Most of the nucellar cells are crushed except those at the chalazal end and a few in the nucellar beak. The contents of the cells of the hypostase lose their granular appearance as did those of the inner integument. In the immediate neighborhood of the vascular strand behind the hypostase are small, densely protoplasmic cells with thin walls. Within this region are six or more branches from the vascular strand, each of which branches again. These branches from the chalaza extend upward around the hypostase so that there are many strands running into the parietal region of the nucellus (fig. 21). These ultimate branches are surrounded by elongate cells which retain their protoplasm. The nucellar tracheids have spirally thickened walls; they extend upward for about a fifth of the length of the ovule.

Other species of Euphorbiaceae.—The species of *Euphorbia* that were examined are closely similar in development. In young ovules, the nucellar beaks of *E. nutans* (fig. 27) and *E. maculata* extend beyond the micropyle before they come in contact with the obturator. In the four remaining species studied, the obturator fills the micropyle and the nucellar beak is short. A similar distinction was pointed out by Schweiger (1905) with reference to certain other members of the Euphorbiaceae.

By the time fertilization has taken place, a central strand is differentiated within the nucellus. It is not well-developed for the region in which it appears it consumed early by the developing embryo sac (fig. 27). Tracheids may be present in the chalaza. Both inner and outer epidermal layers of the inner integument are differentiated in *E. dentata* (fig. 23) and *E. marginata*. In *E. dentata*, striations appear on the walls of the inner epidermal cells of the inner integument of older ovules (fig. 26). In the other species of *Euphorbia* examined, only the outer epidermis, which becomes the stone cell layer, is differentiated. A caruncle develops from the outer integument of each of the six species that were studied as it does in *Acalypha rhomboidea*.

When a 10–12-celled embryo is present, some

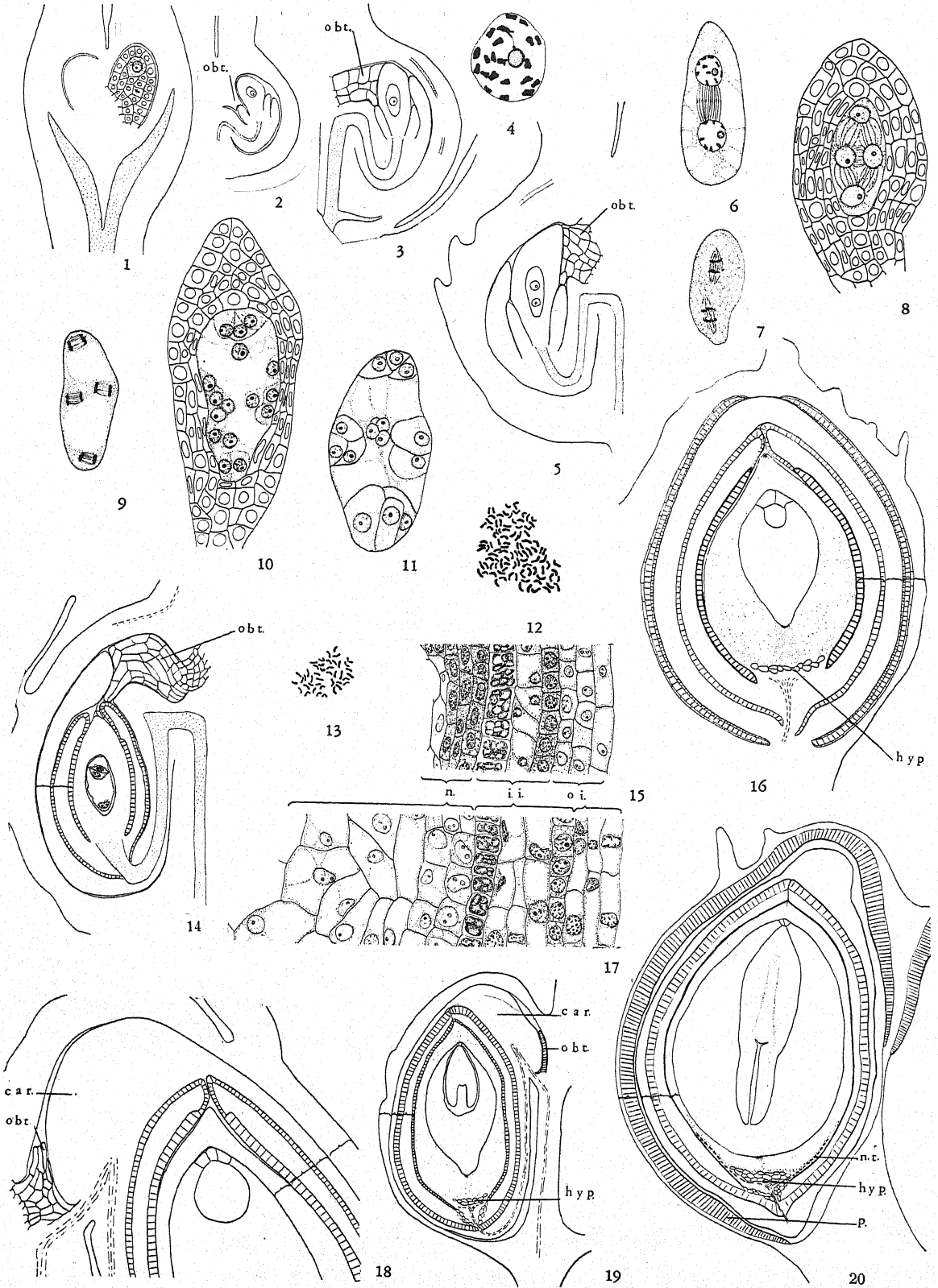


Fig. 1–20.—*Acalypha rhomboidea.* Car., caruncle; c.p., chalazal pocket; c.s., central strand; e., endosperm; hyp., hypostase; i.i., inner integument; o.i., outer integument; n.b., nucellar beak; n.t., nucellar tracheid; obt., obturator;

conspicuous changes have taken place (fig. 24, 25, 28, 29). Cells of the inner integument toward the chalazal end have enlarged at the expense of the nucellus so that the latter appears divided into a chalazal and micropylar portion (fig. 24, 28). The free-nuclear endosperm has encroached upon the nucellus at the chalazal end until it is in contact with the densely staining cells of the nutritive region (fig. 25, 29). The vascular supply terminates in a number of short branches within the chalaza. A number of cells with slightly thickened walls can be seen in the region above the vascular tissue.

Vascular tissue is present in the chalaza of the almost ripe seed of *E. maculata* (fig. 30). A part of the nutritive region still remains between the endosperm and the hypostase. As in *Acalypha rhomboidea* a layer of cells at the chalazal end of the endosperm lacks the visible storage products of the remaining endosperm. Between this layer and the persistent nucellus is a layer of unidentified material. Changes which have taken place in the integuments are those that are characteristic of the family.

The striking feature of *Croton glandulosus* var. *septentrionalis* is the elongate tubular nucellus which extends beyond the micropyle until it comes in contact with the obturator, the tip of the beak being somewhat swollen (fig. 31). When a zygote is present, a central strand is differentiated within the chalazal part of the nucellus. Vascular tissue forms a veritable network in the inner integument, tracheids being present even in the micropylar region. A hypostase of limited extent is present just above the point of branching of the vascular tissue. When the formation of the cotyledons is well advanced, the growth of the endosperm has crushed the inner integument into a thin layer in which the tracheids are conspicuous.

In *Codiaeum variegatum* (fig. 32) the cells of the elongated nucellar beak are arranged in definite rows. The chalazal portion of the nucellus is much more like that in the species of *Euphorbia* examined than that in *Croton glandulosus* var. *septentrionalis*. Older ovules section poorly, but it can be observed that the inner integument increases greatly in size and contains a well-developed vascular supply.

Discussion.—According to Schnarf (1929) corky or lignified cells which appear early in the development of the ovule in the chalazal region of the nucellus are designated the "hypostase." Netolitzky (1926) refers to cells which become differentiated in the same region during later stages of development as chalazal cork tissue. Schnarf (1929) says that it is often difficult to distinguish the two conditions. Johansen (1928) uses the term "hypostase" in the broad sense which Van Tieghem originally intended, namely, to include any of the various modifications which occur in the chalazal region; it is so used in the present paper.

The hypostase first appears in *Acalypha rhomboidea* when the embryo is still spherical and persists as a crushed tissue in the mature seed. It is not found in *E. maculata* until the ovule is nearing maturity. In *Croton glandulosus* var. *septentrionalis*, a few cells are differentiated, just above the point of branching of the vascular tissue in the chalazal area. Thus considerable tissue intervenes between the hypostase and the point at which the inner epidermis diverges from the nucellus.

There is a striking variety of types of vascularization of the ovule within the Euphorbiaceae. In the species of *Euphorbia* examined, vascular strands from the funiculus enter the chalaza where they end in a number of short branches below the hypostase. In *Croton glandulosus* var. *septentrionalis* and *Codiaeum variegatum* there is a veritable network of branching within the inner integument.

p., pericarp.—Fig. 1. Young ovary showing ovule attached in basal position in locule, archesporial cell, and conducting tissue. ×238.—Fig. 2. Somewhat older ovule with macrospore mother cell. Obturator is in contact with the inner integument, which is more advanced than the outer integument "S-" shaped vascular supply. ×138.—Fig. 3. Ovule with macrospore mother cell enlarged. Outer integument more advanced than the inner. Obturator is present. ×138. —Fig. 4. Nucleus of macrospore at diakinesis; 21 pairs of chromosomes. ×1250.—Fig. 5. Ovule after first meiotic division. ×138.—Fig. 6. Macrospore mother cell after first meiotic division; note thickening of spindle fibers. ×510.— Fig. 7. Macrospore mother cell; second meiotic division. ×510.—Fig. 8. Four-nucleate embryo sac with connecting spindle fibers.—Fig. 9. Third nuclear division in the embryo sac. ×510.—Fig. 10. Sixteen-nucleate embryo sac. ×510. —Fig. 11. Embryo sac showing 12 of the 13 cells. ×510.—Fig. 12. Equatorial plate in the endosperm; 97 chromosomes recognizable. ×1250.—Fig. 13. Equatorial plate in root tip cell; 42 chromosomes. ×1000.—Fig. 14. Ovule with two-celled proembryo. Inner and outer epidermis of the inner integument differentiated. Obturator in contact with nucellar beak. Vascular supply ends in the chalazal region. ×138.—Fig. 15. Part of the integuments and nucellus from figure 14. Three cell layers constitute the outer integument. The cells of the outer epidermis of the inner integument are densely protoplasmic, the middle layer is vacuolate, and the cells of the inner epidermis are filled with granular material. Cells of the nucellus densely protoplasmic, with a few disintegrating cells adjacent to the endosperm. ×510. —Fig. 16. Embryo; two of four suspensor cells shown. Stippling shows differentiation within the nucellus; cells of the hypostase are shown at the base. Vascular supply branches below the hypostase. ×138.—Fig. 17. Part of integuments and nucellus from figure 16. The outer integument has three cell layers. The inner integument has an outer epidermis with densely protoplasmic cells, two to three layers of vacuolate cells in the middle region, and an inner epidermis. Peripheral nucellar cells are less vacuolate than those nearer the endosperm. ×510.—Fig. 18. The obturator lies between the pericarp and the caruncle derived from the outer integument. ×138.—Fig. 19. Embryo with cotyledons and suspensor cells. A region of disintegrated cells is present about the embryo. The vascular supply branches below the hypostase. The obturator is crushed between the caruncle and pericarp. ×33.—Fig. 20. Nearly mature seed. Epicotyl, cotyledons, suspensor cells, and vascular primordia of the embryo are shown. Inner epidermal cells of the inner integument are crushed. Vascular branches extend peripherally within the chalazal region of the nucellus. Hygroscopic cells of the second layer of the pericarp are shown. ×33.

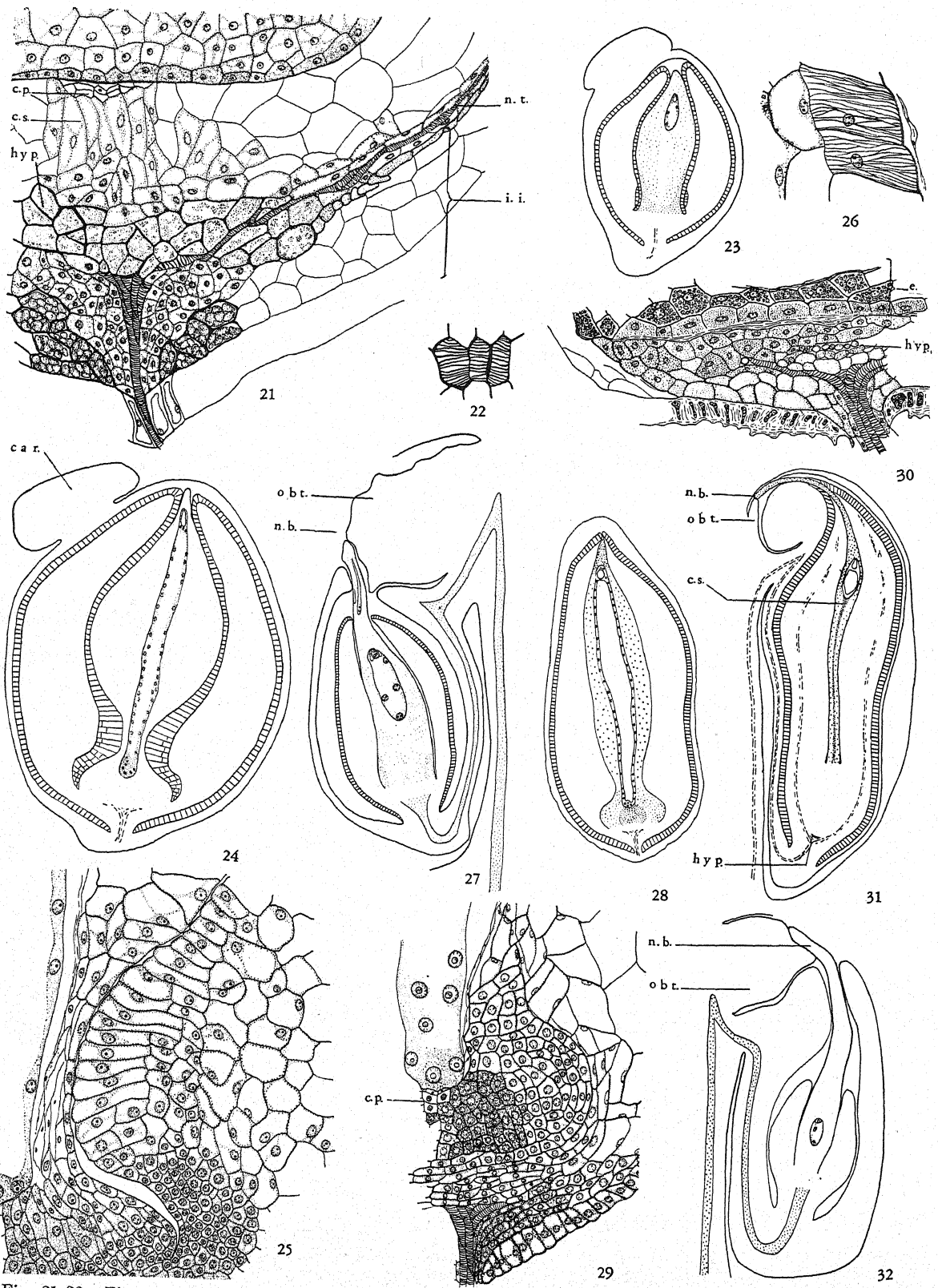


Fig. 21–32.—Fig. 21. *A. rhomboidea.* The vascular supply within the chalaza branches below the hypostase. One of these branches is shown extending upward around the hypostase into the peripheral region of the nucellus. At

According to Kühn (1928) this is the case in *Ricinus communis* and in *Jatropha*. Le Monnier (1872) reported vascular tissue branching freely in both inner and outer integuments of *Aleurites, Dalechampia,* and *Cremophyllum*. In *Acalypha rhomboidea,* short vascular branches appear within the nucellus.

Nucellar tracheids have been reported in several members of other families, the earliest being by Treub (1891) who observed them within the nucellus of two or three species of *Casuarina*. The tracheids were present in the nucellus about the chalazal end of the embryo sac at the time of fertilization. Nucellar tracheids of a similar nature have been described in *Castanea vulgaris* by Benson (1894) and in *Carpinus Betulinus* by Benson, Sanday, and Berridge (1906). Frye (1902) found nucellar tracheids in two ovules of *Asclepias cornuti*. In each case, a single tracheid was observed near the base of the embryo sac at the time of fertilization. These isolated tracheids were not connected in any way with the vascular supply from the funiculus.

An examination of the descriptions of nucellar tracheids in members of the Capparidaceae and Resedaceae by Orr (1921) indicate that he referred to tissues of two different origins. The tissue figured in *Steriphoma cleomides* appears at the micropylar end of the mature embryo sac, and other tracheids are described as present singly or in groups of two or three in the neighborhood of the sac. They are not connected with the vascular supply. These nucellar tracheids seem comparable with those described by Treub, Benson, and co-workers, and by Frye. It is possible that the elements observed are of the same nature as the transfusion tissue in leaves. In mature seeds of other Capparidaceae and of Resedaceae, Orr (1921) found what he called a "tracheal envelope" made up of cells poor in protoplasm with thickenings of various forms on the walls. He considered this envelope to be similar in nature to that described by Guérin (1916) in some of the Thymeleaceae. His figures show cells similar to those present in the inner epidermis of the inner integument of *Acalypha rhomboidea*. It is likely that Orr's "tracheal envelope" is also of integumentary origin.

According to Guérin (1916), nucellar tracheids occur frequently within the Thymeleaceae. In one species each of *Craterosiphon* and *Sinaptolepis,* and in five species of *Dicranolepis,* tracheids, connected with the vascular supply of the funiculus, extend either singly or in groups to the micropylar end of the nucellus. The nucellar tracheids of *Acalypha rhomboidea* and those of the seven species of Thymeleaceae described by Guérin are similar in origin and in their possession of spiral thickenings. In *Acalypha rhomboidea,* however, they are less extensively developed.

Netolitzky (1926) considers the occurrence of tracheids within either nucellus or inner integument to be doubtful and suggests that the tracheids so described are in tissue which has grown up about the endosperm from the chalaza. Kayser (1892) and Schlotterbeck (1896) consider the vascular strands within the tissue about the endosperm of *Ricinus communis* to result from chalazal hypertrophy. Mauritzon (1939) subscribes to this view in his work on the *Thymeleaceae*. No hypertrophy of the chalaza was noted in *Acalypha, Codiaeum,* or *Croton*. Satina and Blakeslee (1941) and Satina (1945) describe the varying degrees of activity of the three germ layers at different times during the development of ovules of *Datura*. Though the major

least one layer of elongate cells lies outside the vascular branches within the nucellus. The inner epidermal cells of the inner integument are flattened and thick-walled. Chalazal cells around the vascular strand have been crushed by the stone cells. Central strand, chalazal pocket and hypostase are shown. A single layer of cells of the endosperm is differentiated in the chalazal region. ×238.—Fig. 22. *A. rhomboidea.* Inner epidermal cells of the inner integument. ×238.—Fig. 23. *Euphorbia dentata.* Ovule with zygote present. Outer and inner epidermal layers of the inner integument are differentiated. The caruncle is shown. Vascular tissue is present within the chalaza. ×56.—Fig. 24. *E. dentata.* A young embryo is present. Free-nuclear endosperm is in contact with the chalazal nutritive region. Inner epidermal cells and cells of the median layers of the inner integument constrict the nucellus into chalazal and micropylar regions. Vascular tissue branches within the chalaza. ×56.—Fig. 25. *E. dentata.* Detail of part of the chalazal region of figure 24. The inner integument is clearly separated from the nucellus. Densely protoplasmic cells of the chalazal region of the nucellus form the nutritive region. Other nucellar cells are vacuolate or crushed. The densely staining antipodal region of the endosperm and the lighter staining more distal portion are shown. ×238.—Fig. 26. *E. dentata.* Striated cells of the inner epidermis of the inner integument. ×238.—Fig. 27. *E. nutans.* Ovule with zygote present. The long nucellar beak with pollen tubes is in contact with the massive obturator. ×138.—Fig. 28. *E. nutans.* A young embryo is present. Stippling shows differentiation within the nucellus. Outer epidermis of the inner integument is differentiated. ×138.—Fig. 29. *E. nutans.* Part of chalazal region of figure 28. The densely protoplasmic portion of the endosperm is in contact in the central region with chalazal pocket which is surrounded by the densely protoplasmic cells of the nutritive region of the nucellus. Crushed and vacuolate cells of the nucellus above the chalazal region adjoin the larger vacuolate cells of the inner integument. The vascular strand, branches within the chalaza. Above the region of branching are horizontally arranged cells. ×238.—Fig. 30. *E. maculata.* Stone cell layer of the inner integument. Vascular strand branches within the chalazal region. The hypostase is present. Remains of the nutritive region persist. The endosperm has a single differentiated layer of cells in the chalazal part. ×238.—Fig. 31. *Croton glandulosus* var. *septentrionalis.* Young embryo and free-nuclear endosperm are present. Central strand is shown in the chalazal portion of the spindle-shaped nucellus. The nucellar beak is in contact with the obturator. Vascular tissue branches within the inner integument. The outer epidermis of the inner integument is differentiated. ×33.—Fig. 32. *Codiaeum variegatum.* Mature embryo sac. The nucellus consists of a long beak and a short chalazal portion. ×56.

part of the nucellus of young ovules develops from the innermost layer, the nucellus of older ovules is derived from the median layer. The innermost layer is then represented only in the vascular tissue of the funiculus. In *A. rhomboidea,* both vascular and sporogenous tissue arise in the region designated morphologically as nucellus. If the innermost layer continued to divide actively instead of slowing down as in *Datura,* it would be possible for more than one germ layer to be represented in the nucellus, one giving rise to the portion in which the embryo sac develops, and the other to the chalazal portion in which the vascular tissue is differentiated. A similar explanation can be made for the occurrence of vascular tissue in the inner integument as in *Croton* and *Codiaeum.*

SUMMARY

In *Acalypha rhomboidea* a single archesporial cell is differentiated while the ovule is in a basal position in the locule. The obturator and both integuments appear between the time of differentiation of the archesporial cell and the appearance of the macrospore mother cell. The mature ovule is anatropous. It is attached near the summit of the locule.

The 16-nucleate embryo sac becomes organized according to the *Penaea* form of the *Peperomia* type—that is, there are four lateral groups of three cells each and a primary endosperm cell whose nucleus is tetraploid.

A hypostase is differentiated in the chalazal region of the nucellus above the vascular tissue before the cotyledons of the embryo appear.

The nucellus is long persistent. When the cotyledons are well developed, numerous vascular branches connected with the conducting tissue of the funiculus are present in the peripheral region of the chalazal part of the nucellus.

In six species of *Euphorbia, E. dentata, E. marginata, E. corollata, E. Esula, E. nutans,* and *E. maculata,* vascular tissue terminates in the chalaza below the hypostase. A nutritive region of densely protoplasmic cells is differentiated in the chalazal region of the nucellus. Nucellar beaks of the two latter species extend beyond the micropyle. Nucellar beaks of the remaining species are short.

In *Croton glandulosus* var. *septentrionalis* and *Codiaeum variegatum,* vascular tissue from the funiculus branches freely within the inner integument. Both species have very long nucellar beaks.

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TITHYMALUS "[TOURN.]" PROPOSED FOR CONSERVATION [1]

Louis Cutter Wheeler

SOME RECENT authors as Rydberg (1932), Small (1933), Hara (1935), and Croizat (1937) have rejected the traditional application of the generic name *Tithymalus* on the ground that *Tithymalus* Miller was earlier, and applicable to a different concept. Bibliographically their grounds are questionable. Nomenclaturally this action, which has already been criticized by Bullock (1938), is unjustified since it is a violation of the resolution of the 1935 International Botanical Congress (Sirks, 1936, p. 361): "As soon as any well-known generic name is found to be endangered, the case should be at once communicated to the Special Committee concerned."

There are two serious objections to the rejection of *Tithymalus* "[Tourn.]" in favor of some other generic name. 1. In the neighborhood of four hundred names have been placed under *Tithymalus* "[Tourn.]" and most of these names properly belong to this concept. (About an equal number of species of *Euphorbia* might still be transferred.) The rejection of *Tithymalus* "[Tourn.]" would allow the transfer of all these species to some other genus which leads to the second objection: 2. The next name to be used instead of *Tithymalus* "[Tourn.]" is likely to be a disputed matter for years to come. Small, and the other authors named above who rejected *Tithymalus* "[Tourn.]", have used *Galarhoeus* Haworth (1812, p. 143). Their only reason for not using *Esula,* which is orthographically simple, was probably the *passé* American Code principle of page-priority. However, Hara (1935) has reduced *Esula* to a synonum of *Galarhoeus* which, under the International Rules of Botanical Nomenclature, ed. 3, Art. 56. (Briquet, 1935), fixes the use. (However, *Esula* Morandi (1761), p. 72, Tab. XL) preoccupies *Esula* Haworth (1812) though this fact has been generally overlooked.) Why *Keraselma* Necker (1790, p. 353, Tab. XXIX, fig. 2) should not have been used by Small and others is not evident. Small (1933) uses *Hydatica* Necker (1790, p. 387) which can only dubiously be related to Tab. XXXI, fig. 14, yet he rejects or overlooks *Keraselma* which can quite certainly be related to and identified by Tab. XXIX, fig. 2, by parallel phraseology. See Wheeler (1943) for discussion of this identification.

Aside from these manifest inconsistencies or inaccuracies of certain authors, the acceptance of Necker's "Species naturales" has been challenged by Wilmott (in Gilmour *et al.,* 1935, p. 92) on the ground that they were not genera. This view is shared by other authors but is not universally accepted. Thus it is evident that while those authors who so far have rejected *Tithymalus* "[Tourn.]" have used *Galarhoeus,* many other authors would very likely use *Keraselma.* Accordingly to reject the traditional genus would allow the transfer of hundreds of species which would result almost certainly in the transfer of many species to both *Galarhoeus* and *Keraselma. Tithymalus* Miller has has already been proposed for rejection in favor of *Pedilanthus* Necker by Bullock (1938) and Wheeler (1939a). The conservation of *Tithymalus* "[Tourn.]" is worth while to preserve usage and avoid the needless labor necessary to catalog the hundreds of combinations likely to be published if it is rejected.

The use of the traditional *Tithymalus* is very interesting as an example of evolution of a generic concept. The pre-Linnaean usage was often, probably prevailingly, in the sense of what is now known as *Euphorbia* L. *sensu latiore.* Yet post-Linnaean usage has been very generally in the sense of all or part of *Euphorbia* sect. *Tithymalus* as used by Boissier (1862, p. 99 et seq.). The use by Hill (1756, p. 148) was the first valid (aside from being pre-occupied) post-Linnaean use of the traditional *Tithymalus.* There were prior post-Linnaean uses of the name in the same sense which were either illegitimate due to the citation of *Euphorbia* L. as an unqualified synonym, or were ambiguous uses. Trew (1754, Tab. 123) validated the genus by reference to several pre-Linnaean works but cited *Euphorbia* L. in synonymy. Scopoli (1754, p. 8) defined the genus in a synoptical key but he has "Tithymalus &c" and the context makes it appear that he probably meant more than one genus. Duhamel (1755, p. 339, Pl. 97) described *Tithymalus* but cited *Euphorbia* L. as an unqualified synonym.

Tithymalus Miller (1754) cannot safely be used even by those who reject *Pedilanthus* Necker because the traditional genus *Tithymalus* was first published (in post-Linnaean times) by Trew (1754, Tab. 123). Both *Tithymali* were published in the same year and *Tithymalus* Miller is not known to be prior and there is no provision in either the International Rules of Botanical Nomenclature, ed. 3 (Briquet, 1935), or the amendments adopted at Amsterdam in 1935, for disposing of simultaneously published homonyms (see Sprague, 1936). Consequently there are no valid grounds for using *Tithymalus* Miller. In spite of this, *Tithymalus* "[Tourn.]" cannot be used without conservation because the authors who published it prior to 1756 invalidated their proposal by citing *Euphorbia* L. as an unqualified synonym (Briquet, 1935: Art. 16, & 60 (1)). According to Art. 61 illegitimate names *can* preoccupy.

In the hope of preventing unnecessary fluctuation in nomenclature it is advisable to conserve *Tithymalus* in the sense of the genus typified by *Euphorbia Peplus* L. Although in the formal proposal which follows, Hill is given as the author, it

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is quite immaterial whether the author be Hill or the earlier Trew. The typification fixes the application of the name. While it is possible to conserve a genus in spite of its having been avowedly superfluous when published, Wheeler has a personal predilection against unnecessarily crediting even a conserved genus to such authorship. There are those who will disagree.

Tithymalus Hill, British Herbal, 148. 1756
versus
Tithymalus Miller, Gard. Dict., ed. 4, Abr. 3: *Tithymalus*. 1754.

Tithymalus Hill. Euphorbiaceae.
Adopted by: Trew, Herb. Blackw., Cent. 2: Tab. 123. 1754; Duhamel, Traité des Arbres 2: 339, Pl. 97. 1755; Hill, Brit. Herb., 148. 1756, Hort. Kew., 172. 1768; Adanson, Fam. Pl. 2: 355, 611. 1763; Scopoli, Fl. Carniolica, ed. 2, 1: 332. 1772; Lamarck, Fl. France 3: 88. 1778; Necker, Elem. Bot. 2: 354. 1790; Gaertner, Fruct. et Sem. Pl. 2: 115. 1791; Haworth, Syn. Pl. Succ., 137. 1812; Rafinesque, Fl. Tellur. 4: 115. 1838; Garcke, Fl. Deutschland, ed. 4, 290. 1858, — ed. 19, 545. 1903; Klotzsch, Monatsb. Akad. Berlin 1859: 251. 1859, Abh. Akad. Berlin 1859 (Phys.): 17, 64. 1860; Dulac, Fl. Dept. Hautes-Pyren., 153. 1867; Karsten, Fl. Deutschl. 2: 121. 1895; Small, Fl. Se. US., 720. 1903; Rydberg, Bull. Agric. Exp. Sta. Colorado St. Coll. 100: 224. 1906 (Fl. Colorado); Millspaugh, Field Mus. Pub. Bot. 2: 304. 1909; Small in Britton & Brown, Ill. Fl. No. U. S. & Canada, ed. 2, 2: 471. 1913; Wooton & Standley, Contr. U. S. Nat. Herb. 16: 145. 1913, Contr. U. S. Nat. Herb. 19: 395. 1915 (Fl. New Mexico); Abrams, Fl. Los Angeles & Vic., ed. 2, 215. 1917; Rydberg, Fl. Rocky Mts., 545. 1917; Britton, Fl. Bermuda, 216. 1918; J. B. S. Norton in Hitchcock & Standley, Contr. U. S. Nat. Herb. 21: 197. 1919 (Fl. Dist. Columbia); Millspaugh & Nuttall, Field Mus. Pub. Bot. 5: 162. 1923 (Fl. Catalina Is.); J. B. S. Norton in Tidestrom, Contr. U. S. Nat. Herb. 25: 342. 1925 (Fl. Utah & Nevada); Prokhanov, Consp. Syst. Tith. Asiae Mediae, 33. 1933; and many other authors and works omitted for the sake of brevity.—About 800 species, *ca.* 400 of which have yet to be transferred here from *Euphorbia:* cosmopolitan.

Type species: *Euphorbia Peplus* L.; Millspaugh (1909, p. 304) made this satisfactory choice of type.

Tithymalus Miller. Euphorbiaceae.
Adopted by: Miller, Gard. Dict., ed. 7, Tithymalus. 1759; Miller, Gard. Kalendar, ed. 14, 208. 1765; Miller, Gard. Dict., ed. 8, Tithymalus. 1768; Miller, Gard. Dict., ed. 6, Abr., Tithymalus. 1771; Small, Man. Se. Fl., 804. 1933; Hara, Jour. Jap. Bot. 11: 381. 1935; Croizat, Amer. Jour. Bot. 24: 702–4. 1937; Croizat, Philippine Jour. Sci. 64: 400. 1938. Miller assigned two binomials to his genus in 1768. The name did not reappear, after Miller's last use in 1771, until 1933 when Small assigned one more species to the genus.

Type species: *Tithymalus myrtifolius* Miller; designated by Wheeler (1939b).

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THE ANATOMY OF THE ABNORMAL SWELLINGS ON THE STEMS OF SOME VARIETIES OF SOYBEANS [1]

B. Esther Struckmeyer

WHILE GROWING several species of plants in the greenhouse in short and long days and at different temperature levels to determine the environmental conditions essential to the induction of blossom buds, a type of response was observed in *Glycine Max* Merr., var. Illini, var. Manchu 3, and var. Minsoy in certain environmental conditions unlike that of plants of any species heretofore studied by the writer. In short days and at a cool night temperature, a swelling or thickening of the third and fourth internodes from the stem-tips and a clustering of short, swollen, and unripened pods at the nodes was characteristic of many plants of these varieties (fig. 1). A limited tendency for this condition to occur in some plants in continuously cool long days has been observed.

Occasionally plants of soybean grown in the field exhibited a similar condition. The internodes near the stem-tip were short and enlarged, the plants often developed little beyond the blossom stage, and when fruits were formed, they were short and stubby. These abnormal plants remained green while the normal ones had mature fruits and had dropped their leaves (fig. 2). It was noted that at least seventy varieties, in breeding and trial plots of the Department of Agronomy of the College of Agriculture, showed this type of abnormal plant. These abnormal plants were quite infrequent except in one variety in which this habit of growth appeared in 10 to 15 per cent of the population. These types of soybean plants are referred to as "duds" by soybean specialists and growers.

Psarev (1940) studied the variety Illini and stated that under the influence of short days the stems reached considerable thickness so that some of the internodes assumed the shape of abnormal swellings or tumors. He concludes that the diameter of short day plants was due to cambial activity, although he does not state whether he was referring to plants with swollen internodes or to normal plants. In either case this is the opposite of what has been found in a study of the anatomical structure of plants during the induction of blossoms (Struckmeyer, 1941). Psarev (1940) does not mention induction in his paper, but we have learned from our observations that this variety when grown in different environmental combinations is an indeterminate type and is induced to blossom in both short and long days. The purpose of this investigation was to study the anatomical structure of the abnormal stems of Illini soybean in comparison with that of the normal stem.

RESPONSE TO PHOTOPERIOD AND TEMPERATURE.—Plants of *Glycine Max* var. Illini were grown in the greenhouses of the Department of Horticulture at the University of Wisconsin. When the plants were approximately 22 days old, they were transferred to the short- and long-day environment in greenhouses maintained at three different temperature levels: 75°F minimum; 65°F day temperature and 55°F night temperature; and 55°F minimum. Plants exposed to short days received approximately nine hours of light daily. The conditions for long days were provided by electric lights. The lights were used from sunset until midnight and delivered approximately 30 to 80 foot candles to the plants. Samples were taken of the swollen internodes and from the fourth internode from the stem-tip of the normal plant approximately four and one-half months after transfer of the plants to the different environmental locations. Dud plants of soybean from the field were sampled at the enlarged region of the stem.

Soybean var. Illini when grown in the greenhouses at Madison does not behave as a short-day plant, as it forms blossom buds in both short- and long-day environments. At a warm temperature (75°F) macrobuds (buds that are visible without magnification) were apparent 18 days after the beginning of the short-day treatment, and 20 days after the long-day treatment. With night temperatures of 55°F and day temperatures of 65°F, macrobuds were observed on plants 30 to 34 days after the beginning of short days and after 35 days when grown under long-day conditions. Plants grown at a temperature of 55°F showed macrobuds after a greater length of time than those given the two previous mentioned treatments. The variety Illini is then indeterminate according to the classification of Garner and Allard (1920), since it flowers in both short- and long-day environments (fig. 4). Manchu is also an indeterminate type. First blossoms of this variety, however, do not abort in the long days as they do with the variety Illini, but set fruit.

The swellings of the third and fourth internode occur in the short days with continuously cool temperatures (55°F), and also in short days with cool nights (55°F) and higher day temperatures (65°F). The swellings or increased diameters of the internodes in short days are not related to induction and fruiting, but to photoperiod and temperature, namely to short days and a cool temperature. Illini soybean is a late variety in the field, not

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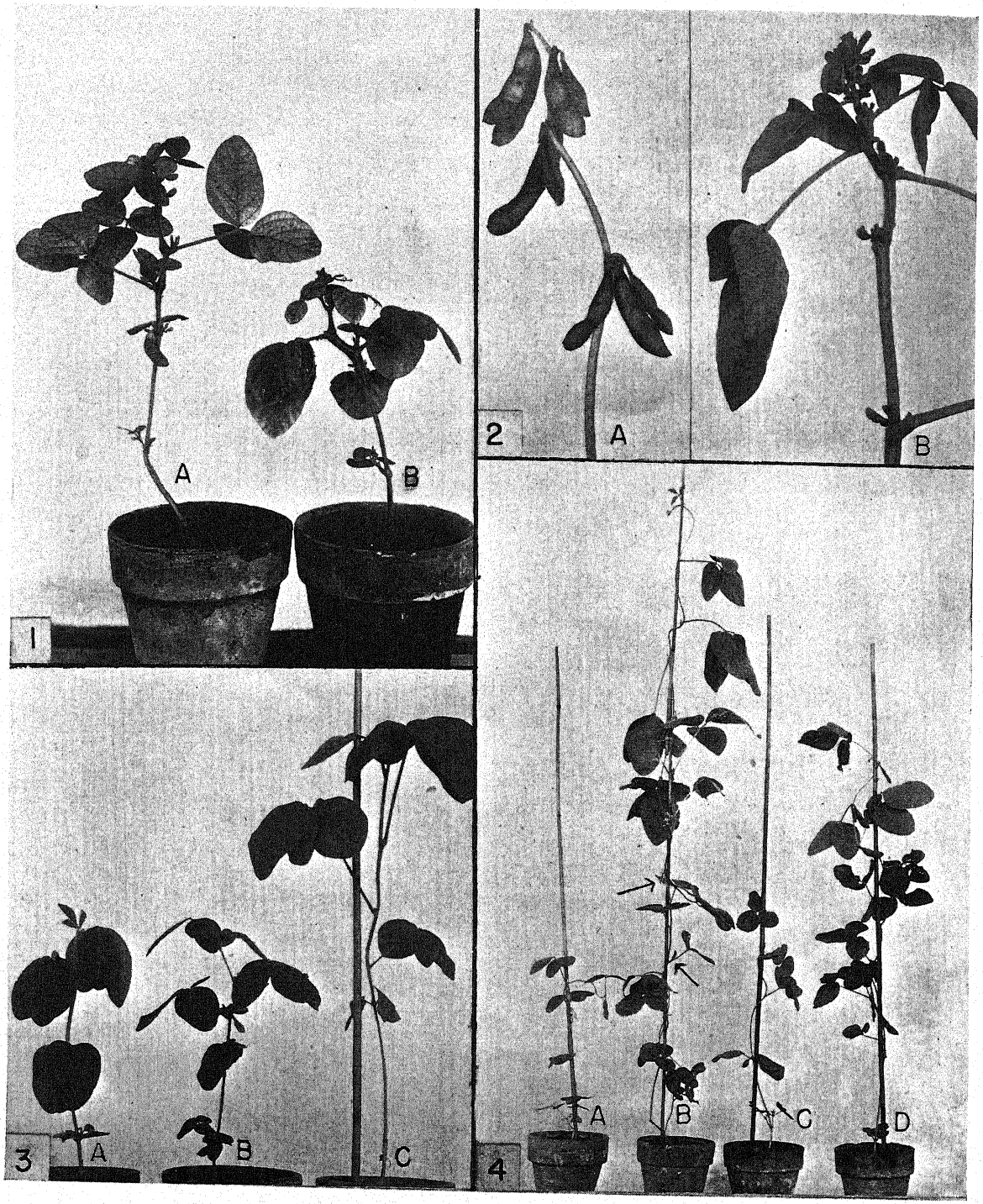


Fig. 1–4. Photographs of normal and abnormal plants of soybean showing their fruiting habits.—Fig. 1. Plants grown in the greenhouse. A. Illini soybean grown in short days and at a cool, night temperature (55°F), showing swelling of the internodes near the stem-tip; B. Manchu soybean grown in the same environment as A showing enlargement of the internodes near the stem-tip.—Fig. 2. Field grown plants. A. Normal plant of Manchu 606 showing natural leaf drop and mature pods; B. "Dud" plant of Manchu 606 of same age as A. The upper internodes are short and thickened; blossoms and fruit are distorted and leaves persist and remain green.—Fig. 3. Young plants of Illini soybean grown in the greenhouse. A. Plant grown in short days and a cool temperature showing epinasty; B. Plant grown in short days and a warm temperature without epinasty; C. Plant grown in long days and a cool temperature without epinasty. Epinasty of the leaves occurs in the environment where swollen internodes are formed.

because it blossoms late, but because early blossoms abort in long days, and it is the later formed blossoms which set and mature fruit.

METHODS.—The samples of the stem were fixed in a Craf (Randolph's Modified Navashin Fluid) fixative (Johansen, 1940). Dehydration with a n-butyl alcohol and infiltration with paraffin were carried out according to the usual method. Sections were cut 12 microns in thickness and stained with iron-alum-hematoxylin and safranin according to the procedure described by Esau (1944).

ANATOMY OF ABNORMAL STEMS.—The stems of plants which showed no swelling had the anatomical structure previously described for soybeans that were vegetative and those induced to flower (Wilton and Roberts, 1936; Struckmeyer, 1941). The anatomical characteristics of a flowering stem are a decrease in cambial activity, a decrease in the formation of phloem and xylem cells, and a thickening of the cell walls of the vascular tissue (fig. 5, 6, 7).

The anatomy of the swollen internode was quite different from that found in the normal stem. The diameter of the swollen internode is approximately three times that of the normal internode (fig. 8, 9). The increase in diameter of the swollen internode results from an enlargement and increase in number of parenchyma cells. The phloem parenchyma cells are greatly enlarged and are very frequently stretched in a radial direction. Groups of early formed sieve tubes and companion cells have been pushed toward the periphery of the stem by the expansion of the parenchyma cells. The first formed phloem fibers appear unchanged. Secondary sieve tubes and companion cells are conspicuous in number and are regularly separated by vascular rays, the cells of which are often large and stretched in a tangential direction (fig. 12). Although there is a relatively wide cambial region, the cells as seen in transverse sections are not the strictly thin-walled, tabular type which is characteristic of cells of normal cambium. Most of them have enlarged and lost their original shape (fig. 11). Secondary xylem vessels are not conspicuous, the majority of cells in the xylem region being tracheids and xylem parenchyma (fig. 12). The number of vascular bundles in the swollen internode is approximately the same as in a normal stem, except that the bundles are much wider because of the tangential spreading of the vascular rays which have forced the phloem groups apart.

The medullary rays are wide, some of the cells dividing and others stretching tangentially, thus separating the bundles to a considerable degree (fig. 10). The pith also contributes to the increased diameter of the stem as the parenchyma cells adjacent to the xylem as well as the xylem parenchyma have increased in number and enlarged in a radial direction so that the protoxylem has been removed some distance from the metaxylem. Only those cells in the center of the pith maintain their normal shape and size (fig. 9). The endodermis may be responsible for a slight increase in diameter as there are some divisions of cells. Endodermal cells have frequently enlarged tangentially between the vascular bundles and fiber groups and divided above them (fig. 14). The epidermal cells, except for enlargement and occasional radial divisions, retain their normal shape. Cells of the cortex also have enlarged but have not increased greatly in number (fig. 14). From a longitudinal section through the normal and swollen part of the stem, it is obvious that the increased diameter of the swollen internode is largely due to an increase in number and enlargement of the phloem parenchyma, to increase in amount of secondary phloem and to a greater width of the cambial zone (fig. 13). The stem increases radially by the enlargement of the pith and phloem parenchyma cells, and tangentially by stretching of the ray cells and enlargement and divisions of the endodermal cells.

Frequently the epidermal and cortical cells become disrupted because of the expansion of the internal tissues. Beneath such an external break is formed a wound cambium (fig. 15). The parenchymatous cells, especially those of the cortex and phloem region, frequently have conspicuous granular inclusions and prominent nuclei.

The anatomical structure of the enlarged areas of dud plants of different varieties from the field is very much like that described for abnormal plants grown in the greenhouse in short days with a cool night temperature (fig. 16, 17). However, an additional interesting feature of the internodes from dud plants was observed. Breaks in the xylem region were quite conspicuous. These breaks, caused by a separation of cells, were such as to give the appearance of cavities in the tissue (fig. 18). These cavities are apparently the result of rapid expansion and enlargement of the pith, ray and phloem cells which exert a tension on the cells which are not enlarging. The break was usually below a group of ray cells, and in several instances stages of tissue differentiation into the space were apparent. The proliferation of new tissue into the space appeared to have had its origin from one of the parenchyma cells of the ray. Various stages of cells differentiating into the space were evident, and

—Fig. 4. Illini and Manchu 3 soybeans planted November 7 and photographed February 8. A. Illini soybean transferred to warm temperature and short days December 1, showed macroscopic blossom buds December 27; B. In long days at a warm temperature, plants transferred December 1, showed macroscopic blossom buds December 30, but the blossoms did not set, later formed blossoms set (arrows); C. Manchu 3 transferred to warm temperature and short days December 1 had macroscopic blossoms December 27; D. Manchu 3 transferred to a warm temperature and long days had macroscopic blossoms December 23. Manchu 3 grown in long days, however, set fruits from early blossoms in contrast to Illini.

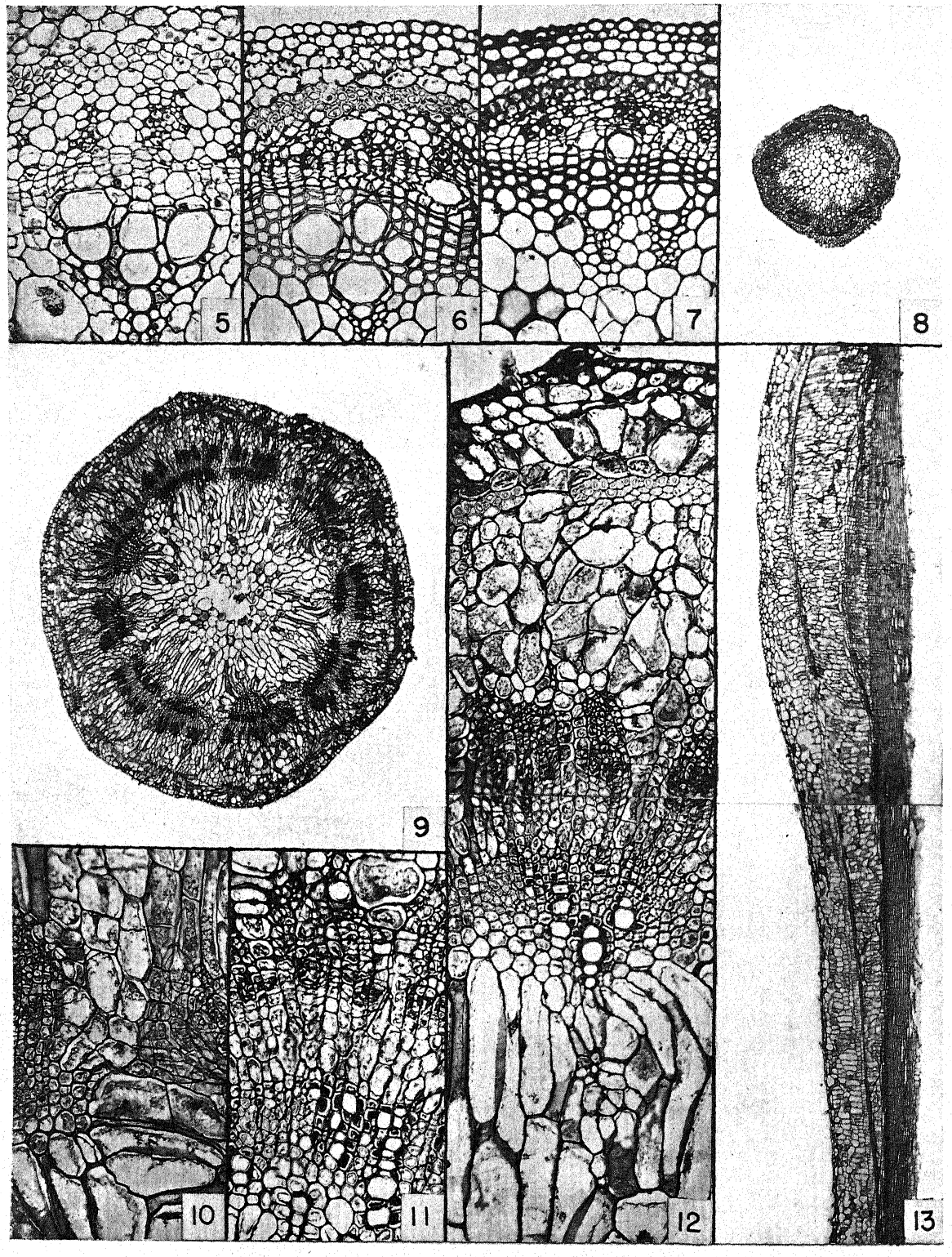


Fig. 5–13. Photomicrographs of normal and swollen stems of Illini soybean.—Fig. 5. Cross-section of fourth internode of young vegetative plant before blossom induction grown at a warm temperature and in a long day. Cambium is conspicuous and is giving rise to phloem and xylem. ×170.—Fig. 6. Cross-section of fourth internode of a flowering plant grown at a warm temperature and in a short photoperiod. Cambium is almost inactive and the adjacent cells have become thick-walled. ×170.—Fig. 7. Cross-section of fourth internode of plant grown in cool

often spaces nearly or entirely filled with cells were apparent (fig. 19).

Thus the anatomy of the swollen internode is quite different from the thick-walled tissue and mature cells of the normal stem.

Discussion.—The response of soybeans to photoperiod and temperature as reported in the literature has been studied primarily with short-day varieties as Biloxi, and not indeterminate varieties as Illini. Withrow (1945) reported a reduced cambial zone in flowering stems of plants of Biloxi soybean and *Salvia*. In our material, the cambium was not actively initiating xylem elements in normal plants that were induced to flower.

Psarev and Neuman (1940) concluded from studies on gross morphology " . . . that the development of xylem in both varieties (Illini and Krushul) was stronger with short days than with long. . . . So it will be obvious that short photoperiods greatly increase the activity of cambial growth in soya." The question arises as to whether a more detailed study of the anatomy of their material would have led to the same conclusion regarding cambial activity.

The anatomical structure of the swollen internodes is quite unlike that of the normal stem, but even in the swollen stem the increase in diameter is not the result of increased cambial activity which results in differentiation of xylem tissue. In this case, growth in diameter was due primarily to the enlargement and increase in number of the cells of the phloem and pith. However, other tissues, to a lesser degree, might account for the increase in diameter of the stem. The dud plants in the field likewise showed anatomical characteristics similar to the greenhouse plants.

The cause of the swollen internodes remains unsolved. It is certain, however, that this condition is not related to flowering as plants grown in short days at a warm temperature and plants grown in long days at different temperatures in which the stems remained normal were flowering. Since greenhouse observations show that the abnormal swelling does occur in short days with cool night temperature, this might indicate that it is a response to some environmental condition. This, however, would not explain the reason for its occurrence in the field where the photoperiod and temperature was unlike that in the greenhouse.

Psarev (1940) observed the swelling of the internodes in short day and suggested its similarity to swellings induced by the application of growth hormones. It is interesting to compare the anatomical structure of the swollen internodes to those described by Kraus, Brown, and Hamner (1936) for stems of bean seedlings treated with indoleacetic acid. The proliferation and differentiation of the cells in the swollen internode does not occur to the extent that is described for the bean treated with indoleacetic acid. Meristematic activity is not as conspicuous in the swollen stem as it is in the treated bean plant, for the increase in size and number of cells seems to be a particularly outstanding characteristic in the former. There are some respects in which the two types of stems are similar. For example, there is an increase in number of cells in the pith, rays, phloem, and to a lesser degree in the endodermis. There is also a general lack of maturing of cells in both the swollen and the treated stems. Another interesting observation, especially on young plants, was the epinasty of leaves on those plants forming swollen internodes at a cool temperature and in short days, especially in dark days (fig. 3). Epinasty of leaves is frequently characteristic of plants treated with a growth hormone. It remains questionable whether or not the swollen internode is the result of a hormone-like substance which manifests itself under certain combinations of environmental conditions.

Perhaps some of the factors, considered by Riker (1942) as possibly being associated with crown gall, would explain the occurrence of these abnormal swellings of the internodes of some soybean plants. It is evident that the swollen internodes, stems of plants treated with growth substances, and pathological tissue, have cells which possess the potentialities for the formation of new tissue and growth.

SUMMARY

Soybean var. Illini responds to photoperiod as an indeterminate type. It blossoms in both short and long days.

The anatomical structure of the fourth internode of normal plants induced to flower is similar to that described for other varieties of soybean and other species of plants.

In short days with cool night temperatures,

temperature and a long day which had aborting blossom buds at the time of sampling. Notice the limited amount of differentiating tissue and the increased thickening of the cell walls. ×170.—Fig. 8. Fourth internode of a plant grown at a cool temperature and in a long day. ×18.—Fig. 9. Cross-section of stem of plant grown at a cool temperature and in a short day. Notice the difference in diameter and cellular structure of this stem compared with the normal (fig. 8). ×18.—Fig. 10. Cross-section of medullary ray from swollen stem. Some of the parenchyma cells are increasing in size and are dividing. ×130.—Fig. 11. Cross-section of cambial region of swollen stem. The cells within the cambial region have enlarged and become distorted and are not typical of cambial cells. ×170.—Fig. 12. Cross-section of swollen internode. The cortical cells have become somewhat stretched. The conspicuous difference, however, is the enlargement of the phloem parenchyma cells which lie below the phloem fibers, the continued differentiation of secondary phloem, and the wide cambial region. Compare with fig. 7. The great increase in size of the pith cells accounts for much of the greater diameter of the abnormal stem. ×130.—Fig. 13. Longitudinal section of normal and swollen region of internode. Notice that enlargement has occurred between the xylem and the phloem fibers. ×18.

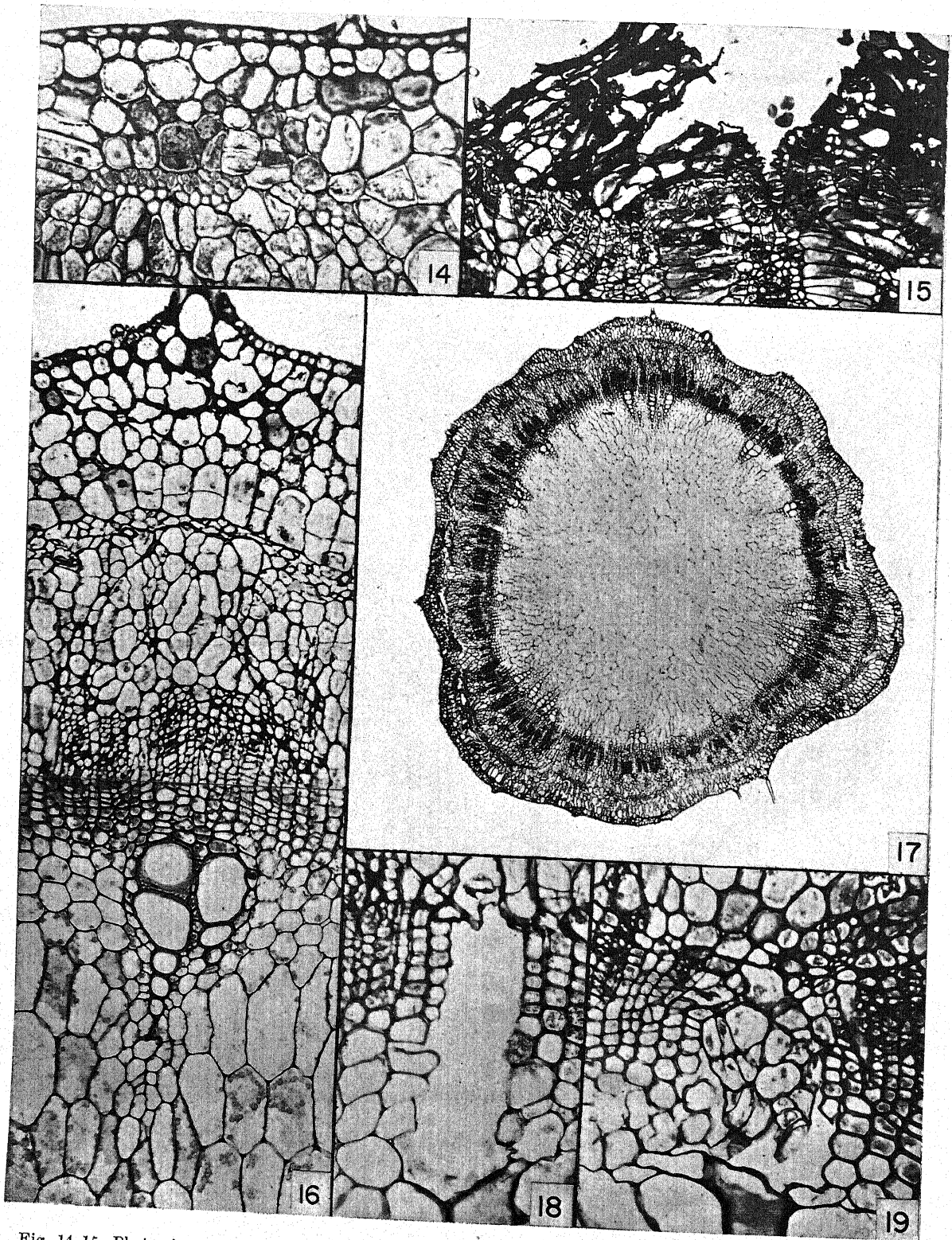


Fig. 14–15. Photomicrographs of swollen regions from plants grown in the greenhouse.—Fig. 14. Cross-section of part of cortical region showing division of the endodermal cells. The cells of the cortex have also enlarged. ×135. —Fig. 15. Cross-section of break in epidermis and cortex of swollen stem. Pressure from increasing cell size and number gives rise to breaks in outer tissue. Wound cambium is being formed below break. ×85.—Fig. 16–19. Photomicrographs of cross-section of dud plant from field.—Fig. 16. Cross-section of dud plant from field. ×18.—Fig.

swellings of the third and fourth internodes occur. These swellings bear no relationship to blossoming, since plants in the long days that are normal also have fruits.

The diameter of the swollen internode is approximately three times that of the normal stem. The increase in diameter of these stems is due largely to the increase in number and enlargement of the parenchyma cells, especially the phloem parenchyma and pith cells. Groups of sieve tubes and companion cells formed by the cambium are frequently separated by vascular rays, the cells of which are enlarged and often stretched in a radial direction. The cambial region is relatively wide but does not consist of the tabular shaped cambial cells as seen in transverse section. The cambium adjacent to the phloem appears to be active in the formation of secondary phloem tissue.

Plants of this nature also were found in the field and are referred to as "duds." When investigating the swollen internodes of these plants, the anatomical conditions are found to be much like those described for abnormal plants grown in the greenhouse. In addition there are distinct breaks in the vascular region of these stems, leaving spaces into which parenchyma cells later are differentiated.

When comparing the structure of the abnormal internodes with stems of other species treated with growth substances by other investigators, it appears that stems treated with growth substances show a great deal of proliferation of cells and meristematic activity, while in the stems studied in this investigation, the increase in diameter is to a great extent the result of an increase in size of the parenchyma cells and to some extent, increase in number of cells.

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17. Cross-section of dud plant from field showing anatomical characteristics similar to those grown in the greenhouse in short days and at a cool night temperature (fig. 9). Cells of the cortex have enlarged, and the endodermis shows divisions. Phloem parenchyma cells have increased in size and number. The cambial region is composed of irregularly shaped cells. Pith cells show enlargement and stretching. ×85.—Fig. 18. Cavity resulting from tension between enlarging parenchymatous cells and lignified tissue. ×170.—Fig. 19. Differentiation of parenchyma cells within cavity. ×170.

NON-LATEX RUBBER IN CRYPTOSTEGIA [1]

J. T. Curtis, R. E. Duncan, and R. Blondeau

THE RECENT article on rubber in *Cryptostegia* by Whittenberger and Kelner (1945) described the occurrence of globules of a hydrocarbon with the properties of low polymer rubber in leaf chlorenchyma cells. A theory was proposed concerning rubber synthesis which was based upon the observation that rubber globules were always associated with chloroplasts in the leaves. The present paper presents observations on the occurrence of similar globules in other parts of the *Cryptostegia* plant.

The studies were conducted on plants of *Cryptostegia grandiflora* R.Br. and the F_1 hybrid, growing both in the experimental plantation of the Societe Haitiano-Americaine de Developpement Agricole at Gonaives, Haiti, and in the botany greenhouses of the University of Wisconsin in Madison. The investigations were made in the period from December, 1942, to February, 1946, on plants varying in age from one year to twenty years. Sections were cut from young leaves, old leaves, whips (Curtis and Blondeau, 1946), wood and bark of old trunks and old roots and were stained with a saturated solution of Calco oil blue NA in 50 per cent ethanol. Globules similar in appearance and in staining and solubility reactions to those described by Whittenberger and Kelner were found in the leaves, old trunks and roots. Those noted in leaves were comparable to the "chlorenchyma rubber" of these authors. There were very few globules or none at all in the young leaves (up to four weeks old) while in old leaves (six months or more in age) the globules were very abundant, occasionally crowding the chloroplasts into one corner of the cell. There was apparently an inverse correlation between the numbers of globules and chloroplasts per cell.

The globules in old woody trunks were confined to secondary parenchyma cells of rays in both wood and bark. They varied in number from 1 to 10 per cell and ranged from 1 to 12 μ in diameter, with the majority 3 μ or less. The smallest globules were thus of comparable magnitude to the largest particles in the latex tubes (Blondeau and Curtis, 1946). They were insoluble in alcohol and acetone but were soluble in xylol and benzene. They stained blue with the oil blue stain. In the absence of a feasible method for the mechanical isolation of globules free from contamination by latex rubber particles, no tests of physical properties or x-ray diffraction pattern could be made. In spite of the absence of this confirmatory information, it is believed that the globules in trunk rays were identical with those described by Whittenberger and Kelner in leaves. Many ray parenchyma cells contained starch grains in addition to globules of hydrocarbon, but no direct association between the two types of inclusions could be detected. Similarly, no relation was found between concentration of globules in the ray cells and proximity of the cells to either latex tubes or to xylem vessels, contrary to the situation reported in leaves, where globules were most numerous in the vicinity of veins. The only distribution pattern noted was a decrease in numbers of hydrocarbon globules in ray cells adjacent to the cambium.

Identical globules were found in xylem parenchyma cells of roots, with a similar size and number range and similar staining and solubility properties. No globules could be found in chlorenchyma cells of young whips nor in other parenchyma cells of these organs. Thus it appears that intracellular inclusions of a hydrocarbon substance similar to rubber are found in storage parenchyma cells of roots and trunks and in certain leaf chlorenchyma of *Cryptostegia* and that these inclusions are correlated with age of cells and not with presence of chloroplasts, since they are absent from young chlorenchyma and ray parenchyma but are present in old cells of both types.

We find no fault with the premise of Whittenberger and Kelner that the basic precursor of rubber is manufactured in chlorophyll containing cells, since a similar statement would be true for nearly all elaborated organic substances in the plant. However, we do not believe that the mere presence of organized globules of rubber in leaf chlorenchyma offers sufficient support for the theory that such globules act as temporary storage centers for rubber which is later digested and translocated in a manner analogous to sugar-starch transformations. The facts that such globules are found in non-chlorophyll containing cells, and occur in greatest number in old cells, and are absent or rare in young, actively photosynthesizing cells, would indicate that the reactions involved are not correlated directly with the carbon assimilation mechanism. Also, the absence of any indication that rubber is digested by intracellular enzymes implies that further investigation is necessary before a definite statement may be made concerning synthesis of rubber *in vivo*.

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ARTIFICIAL AND NATURAL HYBRIDS IN THE GRAMINEAE, TRIBE HORDEAE. II. AGROPYRON, ELYMUS AND HORDEUM [1]

G. L. Stebbins, Jr., J. I. Valencia, and R. Marie Valencia

In a previous paper (Stebbins, Valencia, and Valencia, 1946) artificial and natural hybrids were described involving certain species of *Elymus, Sitanion,* and *Agropyron,* and the cytological results obtained indicated a rather close relationship between these species. In the present paper hybrids are described between two of these species, *Agropyron pauciflorum* (Schwein.) *Hitch.* and *Elymus glaucus* Buckl., on the one hand, and *Hordeum nodosum* L., the commonest perennial species of its genus on the Pacific coast of North America, on the other. Although the genera *Agropyron* and *Hordeum* are usually considered to be rather remotely related to each other, three previous records of natural hybrids between them are known to the writers. The best known is *Agropyron Rouxii* Gren. et Duval of Southern France, which has been interpreted as *A. scirpeum* × *H. maritimum* by Duval-Jouve, and as *A.* sp. × *H. nodosum* by Cugnac (1937). In the Flora of the USSR (1934), Nevski (p. 619) describes *Agropyron Pavlovii* Nevski as a hybrid between *A.* (*Roegneria*) *oschense* Roshev. and *H. turkestanicum* Nevski, and also (p. 721) *Elymus chatangensis* Roshev. as a hybrid between *Agropyron* (*Roegneria*) *macrourum* (Turcz) Drob. and *H.* (*Critesion*) *jubatum* L. The descriptions of these three hybrids and the illustration of *Agropyron Rouxii* correspond rather closely to the *Agropyron-Hordeum hybrids* described in this paper. Like the present one, they are all completely pollen and seed sterile. The only other hybrid known to the writers between a species of *Hordeum* and a member of the series *Triticinae* of the tribe Hordeae is that between *Hordeum jubatum* and *Secale cereale* described by Brink, Cooper, and Ausherman (1944).

The obtaining of the hybrids and the methods of cytological study are as described in the previous paper (Stebbins, Valencia, and Valencia, 1946).

Origin and description of the hybrids.—*Hordeum nodosum* × *Elymus glaucus.*—A single head of *Hordeum nodosum,* no. 401–3, from the second generation of selfing in a strain from Berkeley, was emasculated. The forty florets from this head were pollinated with a strain of *E. glaucus* from Santa Paula (no. 404–2). Two seeds were harvested, which yielded two plants, one of which was maternal in character, and probably resulted from self-pollination. The other, no. 558–1, was intermediate between the two parents in both vegetative and floral characters. It has proved to be very vigorous, and produced flowering heads throughout the summer of 1945. The leaves are bright green, broad and flat, longer than in *H. nodosum,* but not so long as *E. glaucus.* The rachis of the spike is partly fragile, and the nodes nearly always bear three well developed spikelets. The lateral spikelets are nearly or quite sessile, as in *E. glaucus,* while the glumes are aristate, but with a broad basal portion (fig. 2). The orientation of the lowest lemma approaches the ventral position, characteristic of *Hordeum,* but it is somewhat oblique. The palea, on the other hand, resembles that of *Elymus.* As shown in figure 3, the palea of *H. nodosum* is lance-ovate in outline, tapering toward the apex, with its two nerves close together on the dorsal surface. That of *E. glaucus* is narrowly elliptical in outline, with the two nerves lateral and with ciliate keels. The lower palea of the hybrid is essentially like that of *E. glaucus,* but is somewhat tapering toward the apex. The palea of the upper floret of the spikelet, however, is narrower, and its nerves are dorsal on the lower half.

The characters of external morphology in this hybrid agree in general with the description in Hitchcock's Manual of Grasses (1935) of *Elymus aristatus* Merr. Through the kindness of their curators, the writer was able to examine the six specimens of this rarely collected "species" in the U. S. National Herbarium, and the herbaria of the University of California and the Missouri Botanical Garden. Four of these are quite different from *H. nodosum* × *E. glaucus* in their taller, more robust culms, longer spikes with longer internodes of the rachis, glumes completely aristate without broadened bases, and somewhat longer anthers. These four, which include the type collection of *E. aristatus,* are completely sterile as to both pollen and seed; therefore they are probably F_1 hybrids, but not of the present combination. The two other specimens, however, exactly match the artificial *H. nodosum* × *E. glaucus* and likewise are completely sterile. They are from Fulda, Washington, *Suksdorf 5194* (US, MO), and from the "Dead Indian Summit of the Cascades" in Oregon, *Cusick 2958* (US). A third natural hybrid of this combination was found by the senior author in Cottonwood Meadows, Yosemite National Park (no. 3529), where a single plant was growing between its parents.

Agropyron pauciflorum × *Hordeum nodosum.*—This hybrid has been attempted once artificially without success, but the number of florets pollinated (forty) was too few to provide any reliable estimate of its ease or difficulty in nature. The natural hybrid was found growing with its parents in the hills of Contra Costa County, six miles north of Berkeley (Stebbins no. 3387). Here several plants were found in a depression, moist in winter, with a

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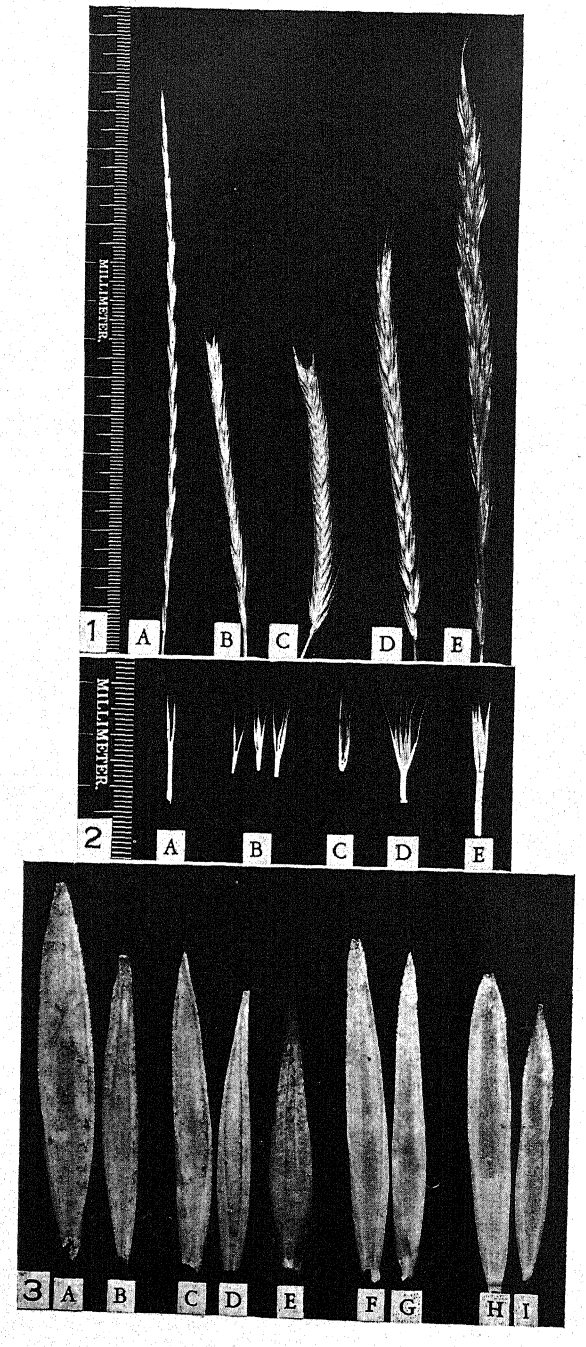


Fig. 1–3.—Fig. 1. Spikes, × ½. A. *Agropyron pauciflorum*, B. *A. pauciflorum* × *H. nodosum*, no. 3387, C. *Hordeum nodosum*, D. *H. nodosum* × *E. glaucus*, no. 558–1, E. *Elymus glaucus*.—Fig. 2. Rachis nodes and glumes, × ⅔. A. *A. pauciflorum*, B. *A. pauciflorum* × *H. nodosum*, C. *H. nodosum*, D. *H. nodosum* × *E. glaucus*, E. *E. glaucus*.—Fig. 3. Paleas, × 5. A. and B. *A. pauciflorum*, from lower and upper florets. C. and D. *A. pauciflorum* × *H. nodosum*, lower and upper florets, E. *H. nodosum*. F. and G. *H. nodosum* × *E. glaucus*, lower and upper florets. H. and I. *E. glaucus*, lower and upper florets.

heavy clay soil. The plants are densely caespitose, with glaucous leaves suggesting those of *H. nodosum*, but narrower and longer. In the characteristics of inflorescence and florets, the plants are intermediate between their putative parents (table 1). In many respects they resemble the artificial hybrids between *E. glaucus* and *H. nodosum*, differing in exactly those characteristics which separate *A. pauciflorum* from *E. glaucus*. Thus the internodes of the rachis are broadly wing-margined in *E. glaucus* and in no. 558–1, while they are narrowly so in *A. pauciflorum* and in no. 3387. The *Elymus* × *Hordeum* hybrid has three spikelets at each node of the rachis, while no. 3387 has one or two. Frequently the spikes of this plant have an intermediate condition, bearing at one node three glumes and two normal lemmas (fig. 2). In two characteristics no. 3387 resembles *H. nodosum* more nearly than does the artificial *H. nodosum* × *E. glaucus*. In *Hordeum* the internode of the rachilla between the glumes and the lemma is very short, while above the single lemma of the spikelet is a prolonged, awn-like rachilla joint. In *Agropyron* and *Elymus*, on the other hand, the internodes or joints of the rachilla between the glumes and the two or three lemmas of each spikelet are more nearly equal, and the relatively short terminal joint bears a somewhat reduced lemma at its apex. In this respect, *H. nodosum* × *E. glaucus* is essentially like its *Elymus* parent, but *A. pauciflorum* × *H. nodosum*, no. 3387, has the terminal rachilla joint elongated, nearly as in *Hordeum*, and bearing a distinctly reduced lemma. A similar resemblance is found in the palea. As explained above, that of no. 558–1 is essentially like that of *E. glaucus*, as well as of *A. pauciflorum* (fig. 3). In *A. pauciflorum* × *H. nodosum* no. 3387 the palea of the lower floret of each spikelet is essentially like that of *A. pauciflorum*, but that of the upper floret is in both its shape and the position of its nerves almost exactly intermediate between those of *A. pauciflorum* and *H. nodosum*.

As an inevitable result of the meiotic conditions to be described below, both no. 558–1 and no. 3387 are completely sterile as to both pollen and seed.

In its external morphology, *A. pauciflorum* × *H. nodosum* no. 3387 fits the description of *Elymus Macounii* Vasey, and specimens from it were identified as *E. Macounii* by Mrs. Agnes Chase of the Smithsonian Institution. The writers therefore examined the fifty-four collections of *E. Macounii* in the three herbaria mentioned above, and found that all were completely sterile as to both pollen and seed. Only five of these, however, resemble closely the authors' collection from north of Berkeley. These are from Sweetwater County, Wyoming, *Nelson 3783* (MO); Yellowstone National Park, *Nelson 6163* (MO); Eastern Washington, *Griffiths and Cotton 535* (US); Malheur County, Oregon, *Griffiths and Morris 872* (US); and Lander County, Nevada, *Hitchcock 10646* (US). The remaining specimens all have longer, more flexuous awns, and

mostly narrower, paler leaves. In these respects they are more like *Hordeum jubatum* than *H. nodosum*. Most of them appear to be hybrids between *Agropyron pauciflorum* and *Hordeum jubatum*, both of which species are common throughout most of the range of *E. Macounii*. There are, however, occasional anomalous specimens, some of them with short rhizomes, which may be hybrids between *H. jubatum* and other Agropyron species, such as *A. repens*. At present, however, the most likely hypothesis is that the specimens classified as *Elymus Macounii* in the current manuals and herbaria are a collection of sterile F_1 hybrids between various species of *Agropyron* and either *Hordeum jubatum* or *H. nodosum*.

CHROMOSOME BEHAVIOR.—*Parents.*—As previously reported by Stebbins and Love (1941) *Elymus glaucus, Agropyron pauciflorum* and the tetraploid form of *Hordeum nodosum* all have the somatic chromosome number $2n = 28$. The chromosome complements of the three species are so similar in their morphological features (form, size, position of the centromere, etc.) that they cannot be distinguished from each other when combined in the hybrids. The description of the meiotic chromosomes and the meiotic process of *A. pauciflorum* and *E. glaucus* have been presented elsewhere (Stebbins, Valencia, and Valencia, 1946). The other parental species, *Hordeum nodosum* (403–1), shows regularly fourteen bivalents at meiosis. No univalents or multivalents were observed. As in *E. glaucus* and *A. pauciflorum* ring bivalents with two or three chiasmata are the most common type. One arm only was paired in 12.3 per cent of the bivalents. The average number of chiasmata is 28.4 per sporocyte or 2.02 half-chiasmata per chromosome, which is a slightly lower frequency than that found in the other two parents. Although meiosis was found to be essentially regular, some irregularities were present at both divisions. At first metaphase one or two lagging chromosomes or chromatids were observed in 2.8 per cent of the sporocytes and one bridge-fragment configuration was found in 8 per cent of the cells recorded. At second anaphase, 5 per cent of the cells showed bridge fragments, but no laggards were observed. Micronuclei were present in 10 per cent of the quartets. In spite of these irregularities, however, the plant is fully fertile.

Hybrids.—The general meiotic behavior is very much the same in the artificial hybrid *E. glaucus* × *H. nodosum* and in the natural hybrid *A. pauciflorum* × *H. nodosum*. In both cases the metaphase pairing is very much reduced and the chromosome separation at anaphase is very irregular. The two hybrids will be discussed separately.

E. glaucus × *H. nodosum.*—Most of the chromosomes (62 per cent) remain unpaired at first metaphase, although only one sporocyte of the forty-one observed showed a complete absence of pairing. The rest of the sporocytes had one to eight bivalents, and associations of three were also present in 54 per cent of the cells. Of the cells with these associations, 73 per cent had one and 27 per cent had two. In addition, one sporocyte showed one chain of four, along with one chain of three, four bivalents and thirteen univalents. The highest degree of association observed, however, was the configuration twelve univalents, five bivalents and two chains of three. The most common configuration (found in 12.5 per cent of the PMCs) was seventeen univalents, four bivalents and one chain of three. The complete analysis of first metaphase is given in table 2. By far the greater number of the bivalents were paired in only one arm, and associations of three were in all cases the simple chain type. Univalents were found irregularly distributed

TABLE 1. *Morphological characteristics of the species and hybrids.*

Species or hybrid		*Agropyron pauciflorum*	*A. pauciflorum* × *H. nodosum*	*Hordeum nodosum*	*H. nodosum* × *E. glaucus*	*Elymus glaucus*
Source		Richmond	Richmond	Berkeley	Artificial	Santa Paula
Plant number		3386	3387	401 (ex 2747)	558–1 (401 × 404)	404 (ex 2865)
Length of spike (cm.)		10–16	9–13	7–8	10–13	13–15
Mean length, internode of rachis (cm.)		0.69	0.39	0.14	0.34	0.55
Margin of internode	shape	narrowly winged	intermediate	broadly winged	broadly winged	broadly winged
	trichomes	short, dense	intermediate	long, dense	intermediate	long, sparse
Number of spikelets per node		1	1–2	1 + (2)	3	2
Lateral florets		0	sessile	pedicillate	sessile	sessile
Number of florets per spikelet		2–3	2	1	1–3	2–3
Length terminal rachilla joint		1.8–2.3	3–3.4	3.5–3.7	1.9–2.2	2.0–2.1
Glumes	Length awn (mm.)	0	2–7	12–13	4–7	0.4–0.7
	Breadth (mm.)	2–2.2	0.6–1.0	0.3	0.9–1.0	1.1–1.3
	Number of nerves	5–6	2–4	1–3	2–3	4–5
Lowest Lemma	Position	lateral	oblique	ventral	ventral-oblique	oblique
	Length of awn (mm.)	4.5–6	6–7	8–9	9–11	13–15
Palea	Shape	bicarinate	weakly bicarinate	rounded, 2-nerved	weakly-strongly bicarinate	strongly bicarinate
	Width between nerves	0.8–1.0	0.5–0.85	0.35–0.45	0.5–0.9	0.7–0.9

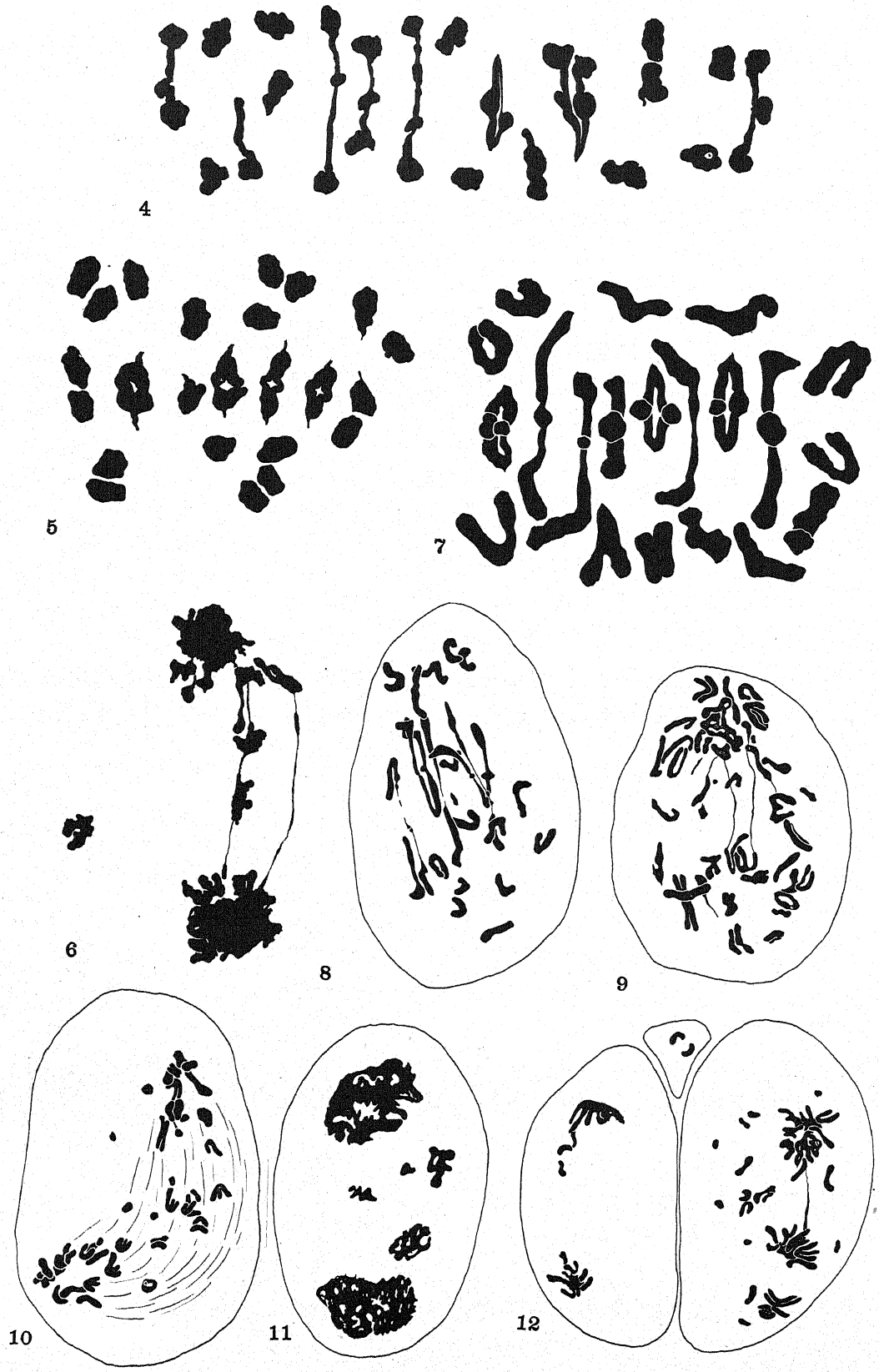


Fig. 4–12.—Fig. 4–6. *A. pauciflorum* × *H. nodosum:* 4 and 5, first metaphase, showing different degrees of chromosomal association; 6, late first anaphase.—Fig. 7–12. *H. nodosum* × *E. glaucus:* 7, first metaphase; 8–10, first anaphase; 11, first telophase; 12, second anaphase.—Fig. 4, 5 and 7, ×1500; figures 6 and 8–12, ×750.

TABLE 2. *Summary of chromosome behavior at meiosis.*

Species or hybrid	Chromosome associations: no. cells	mean number per cell: I	II	III	IV	Chiasmata: no. cells	½ xta per chrom.	xta per cell	I Anaphase: no. cells	% with laggards	% with bridges
Elymus glaucus, 404–2.....	50	0	14	0	0	12	2.2	31.0	77	1.3	0
Agropyron pauciflorum, 403–4..	50	0	14	0	0	15	2.2	30.0	25	0	0
Hordeum nodosum, 403–1.....	30	0	14	0	0	30	2.02	28.4	69	2.8	8
H. nodosum × *E. glaucus*, 558–1	41	17.44	4.24	0.63	0.02	29	0.47	6.55	96	57.2	22
A. pauciflorum × *H. nodosum*, 3387–6	53	15.89	5.57	0.28	0	53	0.51	7.11	50	40.0	84

throughout the cells, usually lying outside the spindle. The chiasma frequency was enormously reduced in comparison with that of the parents, averaging 6.55 per nucleus and 0.465 half-chiasmata per chromosome.

At metaphase the paired chromosomes are oriented in the regular manner, but at late metaphase and beginning anaphase some of them appear abnormally elongated and attenuated on either side of the chiasma. With the advance of the anaphase movement the chromosomes stretch out and form more or less thin chromatin threads, often resulting in fragmentation. It is quite probable that such chromatid bridges are formed as a result of difficulty in unravelling of chiasmata (McClintock, 1941). Other bivalents and trivalents appear to separate normally. At this stage univalents may or may not migrate toward the poles. In many instances they undergo a premature division, the chromatids either joining one of the nuclei or remaining outside as laggards. From one to six laggards (whole chromosomes or chromatids) were recorded in 57.2 per cent of the cells examined and 85.4 per cent showed one to ten fragments of various sizes. Also, from one to four chromatid bridges were found in 22 per cent of the sporocytes; this figure includes bridges due to inversions and those mentioned above which are probably due to failure of unravelling of chiasmata. Only eight sporocytes out of ninety-six showed an apparently clean division at late anaphase.

Other irregularities concerning the distribution of the chromosomes at anaphase are clearly related to abnormalities in the orientation (and formation) of the spindle fibers. In some cells the spindle is curved or elongated; in others it is divided into two parts, forming a tripolar figure and giving rise to three nuclei with unequal chromosome numbers. In a few cells, most of the chromosomes were found to have remained together, forming one large nucleus, while the rest of the chromosomes and fragments were scattered about in the cytoplasm or forming micronuclei. Such abnormalities seem to be due to a failure in the formation of the spindle. Irregularities relating to the spindle were recorded in 16 per cent of the sporocytes.

The first division may result in two, three or four daughter cells, with nuclei of varying size. Always a number of micro-nuclei are present.

The second division is equally irregular in regard to chromosome lagging, bridge formation and fragmentation. Only 8.7 per cent of the anaphases showed normal separation. One to three laggards were found in 69 per cent, one to four fragments in 28 per cent, and bridge-fragment configurations in 37.3 per cent. Micronuclei were present in all the quartets, varying in number from one to fifteen. In addition, many of the sporads contained five to seven microspores instead of the usual four.

A. pauciflorum × *H. nodosum*.—This hybrid behaves cytologically very much the same as *E. glaucus* × *H. nodosum*, but a few differences may be noted. Not quite as many chromosomes were found to be unpaired at first metaphase (57.2 per cent) and no sporocytes were observed showing a complete absence of pairing (see table 2). Chains of three were also considerably less frequent (4.52 per cent of the cells), and of the cells containing these associations only 15.4 per cent had two. No associations of more than three chromosomes were observed. The highest degree of pairing observed is much the same as in the *E. glaucus* hybrid, the lowest number of univalents being twelve. Unlike *E. glaucus* × *H. nodosum*, however, one configuration (sixteen univalents plus six bivalents) was found in very high frequency—i.e., 45.3 per cent of the cells. Chiasma frequency was somewhat higher in this hybrid (table 1). At metaphase the paired chromosomes showed, as in the *E. glaucus* hybrid, abnormal elongation, but no spindle irregularities were seen at anaphase I. The percentage of lagging chromosomes was slightly lower, but chromatid bridges were much more frequent. Second anaphase was not studied.

NATURE OF THE PAIRING.—Chromosome pairing in both hybrids seems to be related to the basic chromosome number of the parents—i.e., seven. The total number of chromosome associations in the metaphases analyzed was frequently seven (although more often six and rarely eight). In *H. nodosum* × *E. glaucus* 39 per cent of the cells examined had six to eight associations, and in *A. pauciflorum* × *H. nodosum* 67 per cent had six to eight. It appears, then, that the pairing may be principally by whole genomes in one of two ways: (a) pairing between one genome of *H. nodosum* and one of *E. glaucus* or *A. pauciflorum*, or (b) autosyndetic pairing between the two genomes of one of the parents. All three of the species involved are tetraploid spe-

cies and therefore contain two genomes of seven chromosomes each in their haploid complements. If autosyndesis explains the pairing, it is probably within *H. nodosum,* since both hybrids show a similar behavior. It is more logical to assume that in both cases the pairing is within *H. nodosum* than to assume that *E. glaucus* and *A. pauciflorum* give the same type of autosyndetic pairing. In addition, it has been pointed out (Stebbins, Valencia, and Valencia, 1946) that autosyndesis within either *E. glaucus* or *A. pauciflorum* is very unlikely. It is not so unlikely in *H. nodosum,* since both diploid and tetraploid forms have been found within this species, as it is currently defined in the manuals.

The evidence at hand is insufficient for determining which of the above possibilities is correct. It has been found (Stebbins, Valencia, and Valencia, 1946) that *A. pauciflorum* and *E. glaucus* differ considerably in the structural arrangement of their chromosomes, including several large translocations. It does not seem likely, therefore, that these two species would give a similar type of pairing with a third species, and they have been found to behave very differently with *Sitanion jubatum.* There is some reason to believe, then, that the pairing in the present hybrids is at least partly autosyndesis between the two genomes of *H. nodosum.* On the other hand, it must be recognized that the structural differences between *E. glaucus* and *A. pauciflorum* might be entirely in the genomes which are not involved in pairing in the *H. nodosum* hybrids. Also, if the pairing in the present hybrids is within *H. nodosum,* it represents considerable homology between the genomes of this species, and therefore quadrivalents would be expected at least rarely in the parental material. None were observed by the writers, although a great many metaphases were examined.

The nature of the associations of three depends upon the nature of the pairing which gives rise to the bivalents. If the bivalents are a result of pairing between the two genomes of *H. nodosum,* for example, then these higher associations must be due to a small amount of homology between *H. nodosum* and the other parent—i.e., auto- plus allosyndesis. If the bivalents are due to allosyndesis between the two parents, then the associations of three must result either from a small amount of allosyndesis within one of the parents (i.e., from auto- plus allosyndesis again) or from structural hybridity. The low degree and irregular character of the pairing suggests that the pairing chromosomes differ considerably in the arrangement of their segments, and that the pairing observed is between homologous segments rather than completely homologous chromosomes. All the associations of three were of the simple chain type which is characteristic of those resulting from structural hybridity. It is obvious that structural differences do exist between the pairing chromosomes, whatever species they belong to, since bridge-fragment configurations are highly frequent at both first and second anaphase, and since rare quadrivalents were observed.

STERILITY.—As might be expected, both hybrids are completely sterile. The sterility obviously is chiefly a result of the extremely irregular meiotic process. The irregularities of meiosis certainly are due in part to chromosomal dissimilarities, causing failure of pairing. It is also quite possible that some of the meiotic abnormalities are genetically controlled. Beadle (1930) has described genes which are responsible for sterility in maize and which cause abnormalities at meiosis, such as curved and divided spindles, very similar to those observed at anaphase in *H. nodosum* × *E. glaucus.* Furthermore, divisions of the *A. pauciflorum* × *H. nodosum* hybrid treated with colchicine behave very differently from similar divisions of the hybrids between *Elymus, Sitanion,* and *Agropyron.* In the former the treated divisions were found by examination of shoot smears to be sectorial chimaeras of doubled and undoubled tissues, and some of the flowering spikes in them were large and coarse. In some of the florets of these larger spikes, furthermore, the caryopses grew to various lengths after anthesis, in contrast to those in undoubled spikes, which never developed at all. In all cases, however, growth of the caryopses ceased after the initial elongation, and they never became plump and filled out, as did those in the doubled sectors of other hybrids. When planted under normal conditions, they failed to germinate.

Cytological examination of meiosis in these chimaeric divisions was not made until late in the season, at which time growth of the caryopses was no longer occurring. This study revealed that most of the microsporocytes had the undoubled number of chromosomes and meiotic behavior similar to that in untreated divisions (fig. 14). In a considerable percentage of them, however, binucleate cells were found (fig. 15), and a few had a single nucleus with the doubled chromosome number (fig. 17), or even associations which suggested the fusion of three originally separate nuclei (fig. 16). In all cases, however, the amount of pairing was the same as in undoubled nuclei; and the general appearance was as if the archesporial cells had divided incompletely, and their nuclei had fused to various degrees before meiosis or during meiotic prophase. First anaphases were very irregular, and characterized by the persistence of the nucleolus (fig. 18). Later stages were not observed.

All of this evidence suggests that in addition to sterility caused by lack of chromosome homology, there is in this hybrid genic sterility associated with abnormalities of spindle behavior and of nuclear metabolism at meiosis. It is probable that fertile allopolyploids cannot be obtained from it. The situation is perhaps comparable to that found by Sears (1941) in the hybrid and amphidiploid between *Aegilops umbellulata* and *Haynaldia villosa.* Before the situation is clear, however, further study is necessary.

RELATIONSHIPS BETWEEN THE SPECIES.—When the cytological evidence presented in this paper is compared with that given in the previous one (Steb-

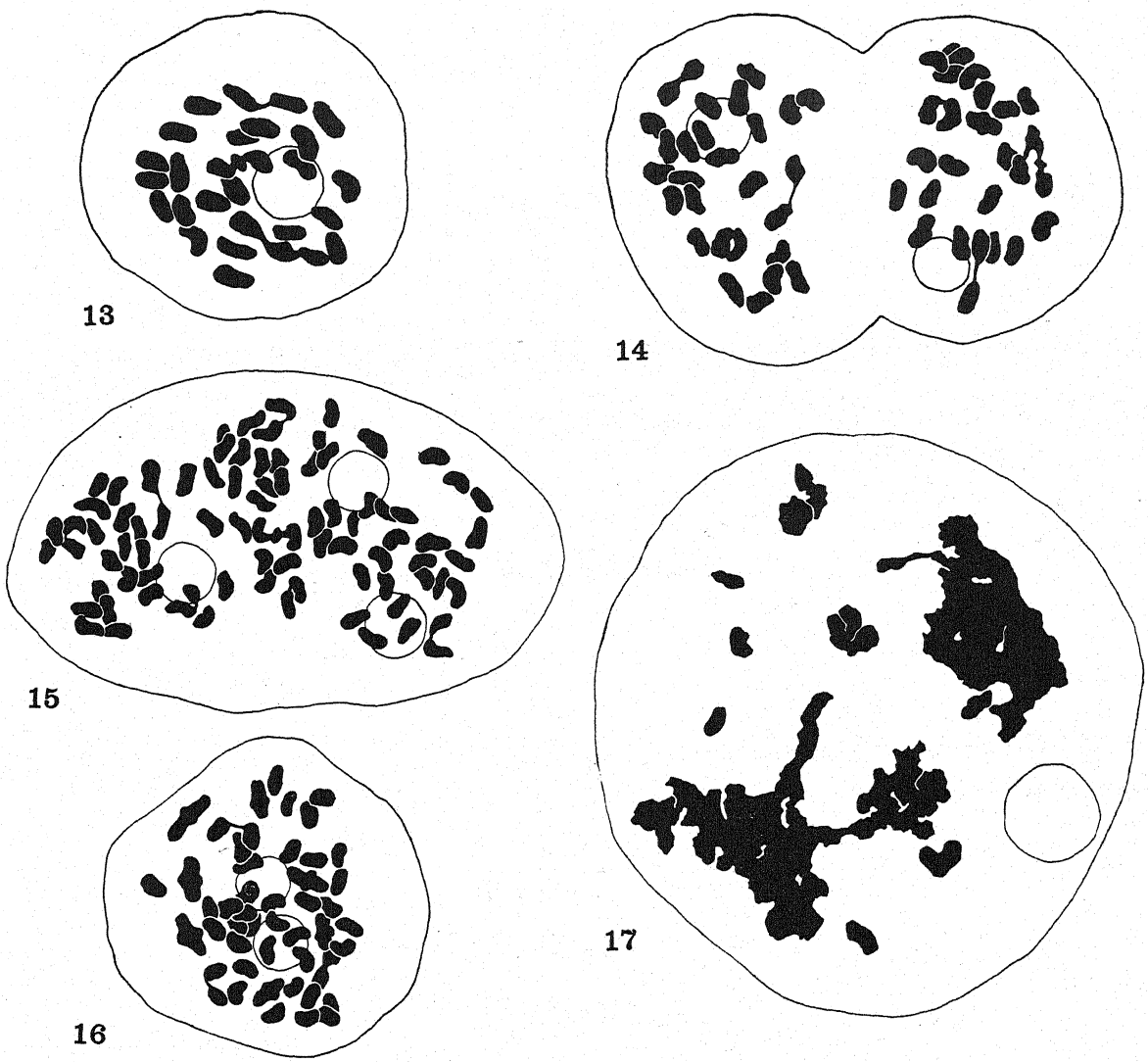


Fig. 13–17. *A. pauciflorum* × *H. nodosum,* sporocytes from division treated with colchicine.—Fig. 13. Diakinesis in normal sporocyte.—Fig. 14. Binucleate sporocyte.—Fig. 15. Sporocyte with three fused nuclei.—Fig. 16. Sporocyte with two nucleoli and 56-chromosomes, at diakinesis.—Fig. 17. First anaphase in sporocyte with doubled number of chromosomes. All figures ×750.

bins, Valencia, and Valencia, 1946), *Hordeum nodosum* is clearly seen to be much more distantly related cytologically to *Agropyron pauciflorum* and *Elymus glaucus* than these species are to each other and to *A. Parishii, Sitanion jubatum,* and *S. hystrix.* If the pairing in the present hybrids is primarily autosyndesis within *H. nodosum,* then this species possesses only a few scattered chromosomal segments homologous with segments of *E. glaucus* and *A. pauciflorum,* and even if the pairing is predominantly allosyndesis, its irregular nature and low chiasma frequency indicates that few if any of the parental chromosomes of the present hybrids are completely homologous. As contrasted with this behavior, all of the hybrids reported in the previous paper have all of the chromosomes paired in at least some sporocytes, and have a high proportion of "closed" bivalents, with two or more chiasmata. It was pointed out in the previous discussion (Stebbins, Valencia, and Valencia, 1946) that the hybrids described in that paper, when compared with those known in *Aegilops* and *Bromus* have the chromosome behavior of hybrids between species belonging to the same section, rather than of intergeneric hybrids. On the other hand, the two hybrids involving *Hordeum nodosum* resemble in their amount of pairing the intersectional hybrids in *Aegilops,* and in the sum of their cytological characteristics are most like the intergeneric hybrid *Aegilops umbellulata* × *Haynaldia villosa.* From the cytogenetic point of view, therefore, the abovementioned species of *Agropyron, Elymus,* and *Sitanion* appear to belong to the same genus, but *Hordeum* may be generically distinct.

This, however, does not fit with the systematic position of these genera as currently recognized. The tribe Hordeae is often divided into the subtribes or series Triticinae and Hordeinae. The first,

which includes *Agropyron, Triticum,* and *Secale,* has the spikelets solitary at the nodes of the rachis, while the second, including *Elymus, Sitanion,* and *Hordeum,* has them in pairs or groups of three. The previous paper pointed out that this character of the number of spikelets at each node of the rachis is not correlated with conspicuous cytogenetic differences. From the present hybrids we can conclude that the morphological differences between *Elymus glaucus* and *Hordeum nodosum* are much more significant indicators of cytogenetic differences than are those between *E. glaucus* and *A. pauciflorum.* Table 1 shows, furthermore, that *H. nodosum* differs from both *A. pauciflorum* and *E. glaucus* in many more characters than the latter two differ from each other. Of the fifteen floral characters listed in table 1, *H. nodosum* is different from the other two in ten, and the three species are about equidistant in three. In only two characters, namely, shape of rachis internodes and number of spikelets per node, are *H. nodosum* and *E. glaucus* similar to each other and different from *A. pauciflorum.* When the vegetative characters are considered, this distinctness of *Hordeum* becomes even more pronounced. In leaf length and width, in the character of the cells of the leaf epidermis, in the auricles at the base of the leaves, and in height and nodal pattern of the culms *A. pauciflorum* and *E. glaucus* are essentially alike, and different from *H. nodosum.* If, therefore, the evidence from external morphology is evaluated on the basis of all of the character differences between the species, rather than on the basis of certain characters which have been traditionally emphasized in systematic treatments, then the morphological and cytogenetic evidence agree closely in pointing to the relatively remote relationship of *H. nodosum* to the other two species.

In this connection, however, the point must be emphasized that the relationship between the three species studied here does not indicate a similar relationship between the genera *Agropyron, Elymus,* and *Hordeum* in their entirety. All three of these genera are complex aggregates of species grouped together on the basis of certain similarities in the structure of the spike or head. *Elymus* is particularly diverse, and contains a number of species which have been placed by some authors in *Elymus,* and by others in *Hordeum.* One species, in fact, was first described by Boissier as *Elymus fragilis,* and a year later was transferred by this same author to *Hordeum.* This and other species described under *Elymus* appear to resemble *H. nodosum* more closely than *E. glaucus.* Until, therefore, the cytogenetic interrelationships of these various species groups, particularly those in *Elymus,* are better known, their generic status must be considered doubtful.

SUMMARY

An artificial hybrid between *Hordeum nodosum* and *Elymus glaucus* resembles some of the specimens referred by systematists to *Elymus aristatus* Merr., and both are completely sterile as to pollen and seed. Similar sterile plants were found in nature along with *Agropyron pauciflorum* and *Hordeum nodosum,* and are intermediate between these two species in external morphology. They are interpreted as F_1 hybrids between these two. They resemble morphologically certain herbarium specimens referred to *Elymus Macounii* Vasey. Because of their complete sterility and their morphological resemblance to the hybrids studied cytologically, all of the specimens referred to *Elymus Macounii* are considered to represent F_1 hybrids between various species of *Agropyron* and either *Hordeum nodosum* or *H. jubatum.* Meiosis in both the artificial *H. nodosum* × *E. glaucus* and the natural *A. pauciflorum* × *H. nodosum* is very irregular. In both hybrids the total number of configurations per sporocyte usually ranges from five to eight. These are mostly bivalents, but one or two chains of three are found in some sporocytes. Twelve to sixteen chromosomes occur regularly as unpaired univalents. The sterility thus is obviously chromosomal to a large degree, but the behavior of plants treated with colchicine and containing sectors with the doubled chromosome number indicates that genic sterility is also present. The cytological evidence indicates that *Agropyron pauciflorum* and *Elymus glaucus* are much more closely related to each other than either is to *Hordeum nodosum.* When all of the differences in external morphology are taken into account, this morphological evidence points in the same direction.

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COMPARATIVE DIFFERENTIATION IN THE AIR ROOTS OF MONSTERA DELICIOSA [1]

Edmund W. Sinnott and Robert Bloch

THE PARADOX of plant development is the origin of differences, often profound ones, among cells which are presumably identical in their genetic constitution. How this differentiation arises, as cells progress from a relatively uniform embryonic state to their specialized mature condition, is one of the major problems of morphogenesis. No solution for it has yet been found, but a careful study of the various phenomena and of particular instances of cellular differentiation may suggest lines of attack upon it and serve to clarify the issues which are involved.

Cells descended from very similar embryonic initials may come to differ in size, form, wall character and physiological behavior. Some differences arise very early, often among the first divisions in embryonic tissue. Thus in axes which grow by means of an apical cell, the successive daughter cells and their derivatives arise in regular sequence and almost from the first differ not only in position but in other characters, so that the origin of the various tissues may be traced in a progressive series of cell lineages. In more homogeneous tissues, the daughter cells from successive divisions may show no visible differences until the last, or almost the last, division. Thus, in the growing surface layer of many roots, the cells which will bear root hairs and those which will not are set apart at the last division, which results in two markedly dissimilar cells. In still other cases, especially in the fundamental tissues, cell differences do not begin to appear until after division has ceased and the cells approach maturity.

The purpose of the present paper is to study the development of an organ where all these types of differentiation occur in order to determine, if possible, what factors may be concerned in the process.

MATERIAL AND METHODS.—Air roots of the aroid *Monstera deliciosa* provide good material for such a study since their tissues show a high degree of cellular specialization but consist of cells laid down in regular longitudinal rows.

Portions of the root were studied from the tip to a point several centimeters back, where the tissues were mature. Both fast and slowly growing roots, and narrow and wide ones, were examined. Material was killed in CRAF and sectioned transversely and longitudinally.

RESULTS.—As in most roots, at the very tip is a complex of embryonic cells from which the regular longitudinal rows of dividing cells, so characteristic of root development, arise. These originate by the early establishment of uniform planes of division which are ultimately transverse to the axis of the root. From the beginning the cell rows near the center of the axis, which will give rise to the stele, are narrower than the more peripheral ones, which form the cortex. Since there is a group of apical cells instead of a single one, it is not always easy to find the differential divisions which set apart the two tissue systems, but such obviously must occur.

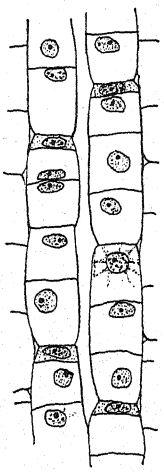
Cell division persists longer in certain cell rows than in others. In the outer or piliferous layer, cells do not cease division until about 3000–4000 micra back of the root apex. The last divisions are often oblique, resulting in alternation between broad-based and narrow-based cells (fig. 3). The latter appear somewhat more embryonic, contain larger nuclei and are the first to form root hairs (fig. 4), though these hairs are ultimately produced by all surface cells under favorable conditions.

Just below the piliferous layer is the hypodermis or exodermis, a single cell layer in which divisions persist to a point about 2500–3500 micra from the apex. All divisions here are equal until the final one which may sometimes occur as close as 2200 micra from the apex and produces two markedly different daughter cells (fig. 3). In each case the basal one (away from the tip) is much smaller than the apical, although the nuclei are approximately equal. Both cells increase considerably in volume before maturity (which is reached about 10 mm. from the apex), but the size ratio between them remains constant (fig. 4). The smaller, possessing a relatively large and more deeply staining nucleus and denser cytoplasm, develops into one of the characteristic "short cells" of the mature hypodermis. The other becomes a much larger cell.

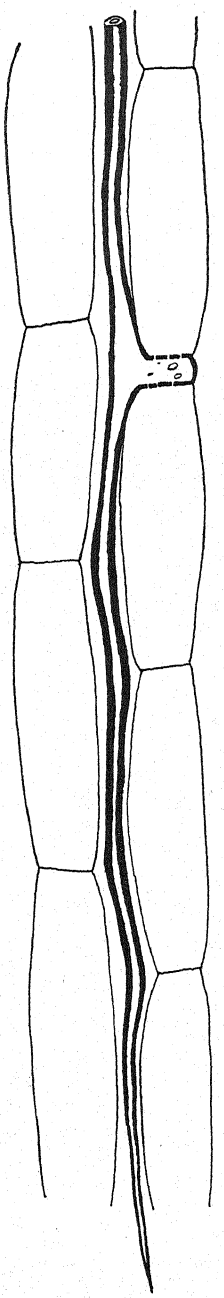
In the cortical tissue between hypodermis and stele, cell division ceases much earlier, at about 1400–2800 micra from the root apex. In the final divisions each cell forms two similar ones and one or both daughter cells again divide, so that a longitudinal series or file of three or four cells is formed, which for a time can readily be distinguished from similar adjacent files. All cells now elongate. In many files of the cortex (though not in all) the last division on the basal side (away from the tip) is unequal and produces a smaller basal daughter cell and a larger apical one. These unequal divisions may occur as close as 700 micra from the root apex and continue for some time. The file as a whole thus usually consists of three larger cells with a terminal smaller one at the basal end (fig. 1). Not infrequently the small cell divides into two.

This tissue thus resembles the hypodermis in the basally polarized and unequal character of many of its last divisions, which result in two cells, different in size but with approximately equal nuclei. Here, however, the small basal cells develop very differently from similar ones in the hypodermis, for they grow into the remarkable long and branching trichosclereids which form a mechanical system in the

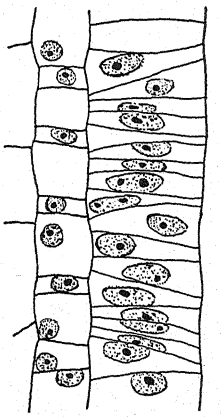
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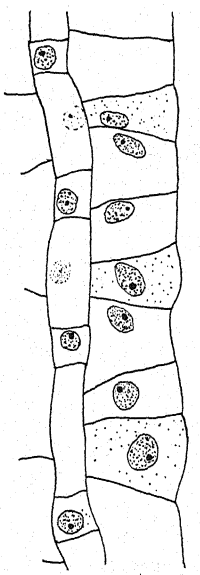
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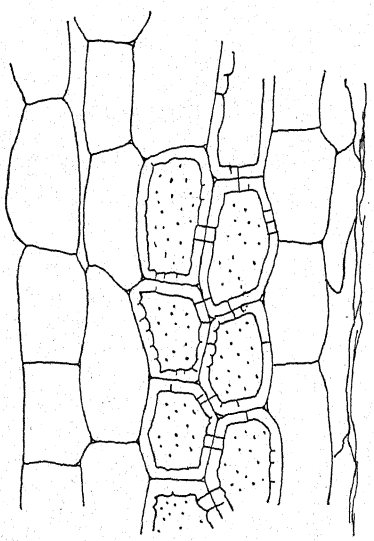
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Fig. 1–5.—Fig. 1. Longitudinal section through root meristem, 800 μ from apex, showing formation of five trichosclereid initials at basal (proximal) end of cell files in cortex. One nucleus is in prophase. ×315. (In all figures the root apex is toward the bottom of the page.)—Fig. 2. Fully differentiated trichosclereid in mature region of cortex, 5 cm. from apex. ×315.—Fig. 3. Longitudinal section, 3500 μ from apex, showing piliferous layer (right) and hypodermis (left). ×315.—Fig. 4. Similar section through a slightly thinner root, 10 mm. from apex, showing beginning of root hair formation in piliferous layer (right) and almost fully differentiated hypodermis (left). ×315.—Fig. 5. Longitudinal section through outer cortex of air root 6 mm. in diameter, showing differentiation of lignified brachysclereids below surface (right). ×230.

cortex of these air roots (fig. 2). Their development has recently been described by Bloch (1946).

Still farther back on the root (about 3–4 cm.), after the root hairs and piliferous layer have disappeared, cells in the relatively homogeneous parenchyma of the cortex may develop thick walls and differentiate directly into brachysclereids (Bloch, 1944). These appear in the inner cortex, abutting

directly on the endodermis, and in the outer cortex, under the hypodermis (fig. 5). At this level the trichosclereids also become thick walled and reach their mature condition (fig. 2).

Discussion.—In the hypodermis and the rest of the cortex and to a limited extent in the piliferous layer, differentiation thus visibly begins at certain of the last divisions and the resulting pairs of unequal cells are very similar. Why is it, one may ask, that in the further differentiation of these pairs of cells such dissimilar elements as hypodermal short cells and trichosclereids are formed? Clearly this is related in some way to the spatial and temporal position which these cells occupy in the developing system, the cortical initials lying nearer the center of the organ and appearing earlier than those of the hypodermis.

The immediate environment of the two cell types provides a difference which may be related to their different development. The cells of the hypodermis are closely in contact with their neighbors and air spaces around them are lacking. The rest of the cortex, however, is well provided with intercellular spaces. These are at first usually triangular (as seen in cross section) but as cell contacts decrease, spaces may coalesce and become much larger. They extend for long distances longitudinally. It is into these spaces that the processes from the sclereid initials grow, and the size, shape and course of these processes seem to be determined by the topography of the space system available to them. The fact that only the small basal cells of the files grow out into these spaces seems to be due to the more "embryonic" character which these cells possess because of their denser cytoplasm and the relatively large size of nucleus in proportion to cell. When the final division is more nearly equal, as sometimes happens, a sclereid is not formed, indicating that the presence of a small embryonic cell is necessary for its development. Such a cell in the hypodermis cannot enlarge because there are no spaces into which processes may grow. In the cortex, however, or in the piliferous layer directly at the surface, where adjacent spaces occur, such processes may readily be formed. These trichosclereids essentially are internal hairs, and in their development somewhat resemble root hairs which (in many cases) also arise from the smaller of the two daughter cells of an unequal and polar division (Sinnott and Bloch, 1939).

Differentiation in these cell types (hypodermal short cells, trichosclereids, and their sister cells) thus seems to involve two elements: first, an unequal division which at once sets off cells markedly different from each other, the smaller being inherently capable of much more complex development than the larger; and second, a difference in position or environment, which results in further differences, during later development, between the otherwise very similar small daughter cells.

The brachysclereids of the cortical parenchyma, however, which differentiate still later in the cortical tissue do not owe their origin to visible differences at cell division, for they may appear some time after division has ceased and in fundamental tissue where the cells seem all alike. They develop at the time when the walls of the long trichosclereids thicken and mature. Their position at the inner and outer portion of the cortex suggests that environmental relations may be important in determining their differentiation. This idea is supported by the results reported by Bloch (1944) who found that when a new surface is made by cutting away a portion of the root, a layer of these short sclereids develops a little way below this surface, from cells which in a normal root would have formed thin-walled parenchyma only. Such redifferentiation of thick-walled cells below wound surfaces is especially significant in air roots of orchids (Bloch, 1935) where there is no late cell division at all and no wound cork is formed. Apparently the formation of a sclerotic wall in a parenchyma cell is related to the position of this cell with reference to particular environmental factors, such as water relations, a surface-interior gradient in oxygen or pH, or other factors (Bloch, 1941, 1944). All cells here have apparently the capacity to form sclerified walls, but only those in a particular position in the physiological system actually do so.

One may, therefore, distinguish two main factors concerned in cellular differentiation, illustrated in the air roots of *Monstera* and in the histogenesis of plant structures generally.

First, there is the polar character of cell division. This may be obvious, as in the cases here described, where cell polarity is parallel to the axis of the organ and results in unequal cytoplasmic distribution and in cells dissimilar in size. In other cases, which result in the setting apart of stomatal initials or similar specialized cells, the orientation of the polar axis may be more variable and cell inequalities of a more complex sort are produced. The directions of cell expansion, and thus cell shape, are usually expressions of polarity. Often the only visible result of cell polarity is the plane in which division occurs. In all such cases, the specific polarity of a cell seems to be related to the position which the cell occupies in a developmental pattern. The original polar axis may sometimes be directed by factors in the external environment, as has been shown for the egg of *Fucus* and similar material; but once this axis has been established, its various sub-axes and the polarity pattern of the structure in general seems to be a true "unfolding" and under the control of inner factors, ultimately determined by the genetic constitution of the individual.

Many differences in the size, shape and constitution of cells result from such polar divisions and are thus related to the position of a cell in a specific polarity pattern. Many others, however, seem more closely related to the position which the cell occupies in a specific physiological system, or on an intensity gradient of one or more factors in the external or internal environment. Many or all the

cells of a growing structure seem to possess an equal range of reactivity or developmental potency, and the particular fate of each cell, the specific differentiation which at a given stage it will undergo, depends on an environmental stimulus of a particular kind or degree, as is illustrated by the development of brachysclereids in the cortex of *Monstera*. Experiments in regeneration have shown that in many other cases differentiated cells typical of epidermis, hypodermis, endodermis, cork or xylem may be formed as the result of changed environmental relations, in tissues which otherwise would have very different fates.

Usually both these factors (polarity and environmental influences) are concerned, as is the case in the differentiation of trichosclereids and short hypodermal cells in *Monstera*. In a sense every cell division may be looked upon as differential, since its two daughter cells differ in position if in nothing else; and in a sense the environment is effective in the unfolding of a pattern, since it provides the physiological setting in which development takes place. But for a precise investigation of the factors which govern any particular case of cellular differentiation, it is useful to recognize these two major influences. It has well been said that "the fate of a cell is a function of its position"; but it is important to distinguish between position in particular environmental or physiological gradients and position in a more recondite developmental field or pattern, set up by a configuration of polar axes. This contrast is part of the more general antithesis between environmental and genetic factors which meets us everywhere in morphogenetic problems.

SUMMARY

In the air roots of *Monstera deliciosa* it is possible to distinguish several types of cellular differentiation.

As in most roots, early differential cell divisions at the apex set apart the main tissue systems.

All the final divisions in the hypodermis and many of those in the rest of the cortex are polar and each results in two markedly different daughter cells, the apical larger and more vacuolate, the basal smaller and more densely cytoplasmic. Further differentiation of these smaller cells results in mature elements which are very dissimilar, those in the hypodermis forming the characteristic short cells of this layer, but those in the cortex becoming long, branched and thick-walled trichosclereids. This difference seems to be related to the presence of well developed air spaces between cells of the cortex which are absent in the hypodermis.

Considerably later, certain parenchyma cells of the cortex may become thick-walled brachysclereids. Experiment shows that this differentiation depends on the relation of these cells to a surface (either natural or the result of wounding).

The fate of a cell thus seems to depend (1) upon its position in an unfolding temporal and spatial pattern of development, related primarily to a system of genetically determined cell polarities, and (2) upon its position in a physiological system, related primarily to environmental factors.

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THE INFLUENCE OF THE COMPOSITION OF THE MEDIUM ON GROWTH IN VITRO OF EXCISED TOBACCO AND SUNFLOWER TISSUE CULTURES [1]

Albert C. Hildebrandt, A. J. Riker, and B. M. Duggar[2]

PATHOLOGICAL CELL proliferation in plants is of special interest because of the possible relationship to diseased growth in animals and man. Therefore, the crown-gall disease incited by *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.* has been studied extensively. The early literature on crown-gall has been reviewed by Riker and Berge (1935) and by Levine (1936). Although much work has been done with crown-gall bacteria, little is known yet about the physiology of the host cells, because of certain technical difficulties involved in such studies.

The plant tissue culture technique offers certain opportunities for studies of tissue metabolism not possible with intact plants. For example, isolated cell masses may be cultured *in vitro* on a medium which, except for traces of impurities, contains only known chemicals. There are no neighboring cells, tissues, and organs to influence development. The tissue cultures either grow or fail to grow because of the materials originally either absent from or present in the medium and because of the tissue metabolites.

The influences of temperature, pH, sucrose concentration, certain plant extracts, and crown-gall bacterial products on growth of plant tissue cultures *in vitro* have been reported earlier (Hildebrandt, Riker, and Duggar, 1945, 1946). Certain of these extracts at low concentrations caused increased growth of tissue cultures, but often they inhibited growth of the cultures at higher concentrations.

Since certain extracts stimulated growth of tissue cultures, this stimulation might have been due either to the introduction of more favorable quantities of certain of the constituents of the basic medium or to factors not present in it. For example, certain preliminary experiments suggested that higher concentrations of iron resulted in better growth of the tissue cultures. Therefore, the influence of various concentrations of salts and vitamins in the basal medium on growth of tobacco and sunflower tissue cultures was studied in three salt solutions. A report on part of the work herein reported has already appeared in an abstract (Hildebrandt, Riker, and Duggar, 1944).

MATERIALS AND METHODS.—The materials and methods were essentially the same as those described earlier (Hildebrandt, Riker, and Duggar, 1945). Tobacco and sunflower tissues were used. The tobacco tissue was from the hybrid *Nicotiana glauca* Grah. ♀ × *N. langsdorffii* Weinm. ♂ and was isolated and supplied by Dr. P. R. White. The second tissue was isolated from "secondary" crown galls on sunflower (*Helianthus annuus* L. var. Giant Russian). Both tissues were free of crown-gall bacteria and were capable of unlimited growth *in vitro*.

The composition of the basal medium (White, 1942) is repeated later for comparison with improved media. Reagent grade chemicals, except sucrose, ferric tartrate, boric acid, and potassium iodide, were used, and a record was kept of the impurities in the salts. The agar was leached by washing in distilled water at least 3 times daily over a 3- to 4-day period. All experimental cultures were incubated in 125 ml. Erlenmeyer flasks containing 50 ml. of medium. The flasks were washed previously in potassium dichromate-sulfuric acid cleaning solution and rinsed in tap and distilled water.

The experimental cultures were maintained in the dark at 26° ± 1°C. in a special culture room. Twenty-four tissue pieces (four per flask) were used per treatment, and each experiment was repeated three times unless otherwise noted. To determine the amount of growth the tissue pieces were removed from the flasks after a 6-week incubation period and weighed individually. All data were analyzed statistically to determine significance between treatments.[3]

Media prepared according to the triangular method were used to study the influence of increasing and decreasing concentrations of each of the constituents of the basal medium. Ferric tartrate, ferric sulfate, and thiamine hydrochloride were sterilized by filtration; all others were autoclaved. The three salts under test were added aseptically to the culture flasks to avoid precipitation. The growth on nineteen different media of representative sunflower tissue and the concentrations of the three salts under test from one such experiment are shown in figure 1, A and B, respectively, as an example of the method. A tissue grown on the unchanged basal control medium is shown at the upper left of the triangle; tissues grown on media without one of the three salts under test are shown at the upper right of the triangle (fig. 1, A). The omission of one nutrient did not mean necessarily that those materials were lacking completely because, for example, of impurities in the washed agar, the salts, the sugar, and in the carry-over of various nutrients with the seed tissue pieces, which were grown on the basal medium.

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[2] The writers are indebted to Eugene Herrling for preparing figures 1 and 2.

[3] The authors are grateful to Dr. J. H. Torrie for his counsel in connection with the statistical analyses.

The three salts under test are indicated at the outside corners (fig. 1, B). In successive rows the concentrations of each salt were doubled. For example, the culture at the upper angle of the triangle was grown on a concentration of ferric sulfate of 0.0025 g. per liter; the next two cultures in a horizontal row grew on media with ferric sulfate concentrations of 0.005 g. per liter; the three in the next lower row grew on media with concentrations of 0.01 g. per liter; the four in the next row grew on media with concentrations of 0.02 g. per liter; and the bottom five tissues grew on media with concentrations of ferric sulfate of 0.04 g. per liter. final pH values of the media, respectively, were 4.6, 5.1, 5.7, 4.6, and 4.2. There was also a progressive increase in acidity in cultures containing 0.0025, 0.005, 0.01, 0.02, and 0.04 g. per liter of ferric sulfate, respectively, which averaged pH 5.6, 5.5, 5.4, 5.2, and 4.8 after incubation.

EXPERIMENTAL RESULTS.—This method was employed with tobacco and sunflower tissue cultures to study the effects of different concentrations of all the constituents of the basal medium plus ferric tartrate, nicotinic acid, and pyridoxine. The results for sunflower tissue cultures are presented in figure 2. The results with tobacco cultures with slight

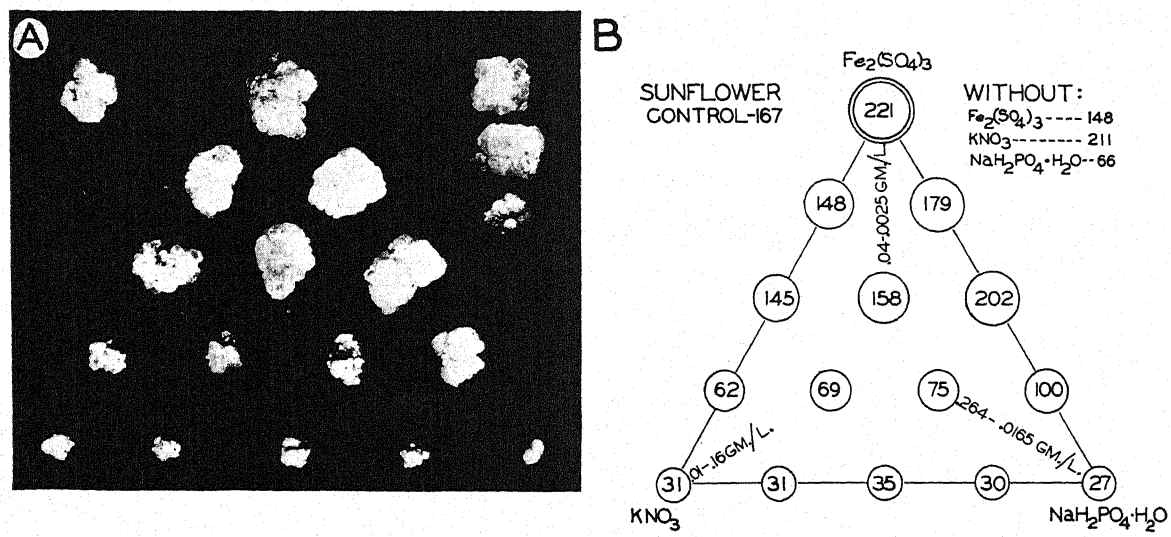


Fig. 1. Examples of the triangular method showing the influence of increasing and decreasing amounts of ferric sulfate, potassium nitrate, and sodium dihydrogen phosphate on growth *in vitro* of sunflower tissue cultures.—A. Actual representative sunflower tissue cultures grown on each of the 19 media described in B and arranged in the same order.—B. The average wet weight in milligrams (of 24 tissues grown on each medium in a single experiment) is indicated inside the circles, which roughly vary in size corresponding to the amount of growth. The medium considered best has a double circle. The weight of the tissues on the basal medium is given at the upper left of the triangle. Those on the basal medium from which was omitted each of the salts being studied are indicated at the upper right of the triangle.

These concentrations appear as 0.04 and 0.0025 g. per liter inside the apex of the triangle. The same procedure was applied to concentrations of potassium nitrate from 0.01 to 0.16 g. per liter and of sodium dihydrogen phosphate from 0.0165 to 0.264 g. per liter. Representative tissue cultures grown on the nineteen different media are shown in figure 1, A; the average wet weight of twenty-four tissue cultures grown on each of these nineteen media is shown within the circles in figure 1, B. The double circle indicates the medium considered to contain the best concentrations of the three salts under test. Thus, the largest tissue culture at the top of the triangle was grown on a medium containing, respectively, 0.0025, 0.16, and 0.264 g. per liter of ferric sulfate, potassium nitrate and sodium dihydrogen phosphate. Usually the inhibiting effect of ferric sulfate was associated with an unfavorable hydrogen-ion concentration. For example, in five cultures containing 0.04 g. per liter of ferric sulfate, the modifications from an earlier report (Hildebrandt, 1944) are shown in figure 3. The concentrations of the various salts were doubled in successive rows as above, except with tobacco cultures (fig. 3, F) where the concentrations of thiamine hydrochloride, pyridoxine and nicotinic acid were tripled. In triangles F (fig. 2 and 3), pyridoxine and nicotinic acid were vitamins not present in the basal medium. In certain cases the double circle is not around the greatest weight. For example, in figures 2, C and 2, D, the average wet weight of cultures on the media considered to have the best concentrations was less than others. In such cases these concentrations were considered best because in each of the three runs these particular media gave consistently good growth, whereas other concentrations showed a higher average wet weight due to especially good growth in just one run. In the other two runs growth was definitely below average. Each number represents the average wet weight of 72 tissue pieces

from experiments (24 cultures in each experiment) run at three different times, except in figures 3, A and 3, B, where each number represents the average wet weight of 84 tissue cultures from four experiments and in figure 3, F, where each number is the average wet weight of 60 tissue pieces from 3 runs. The final acidities of all culture media were determined at the end of the six-week incubation period

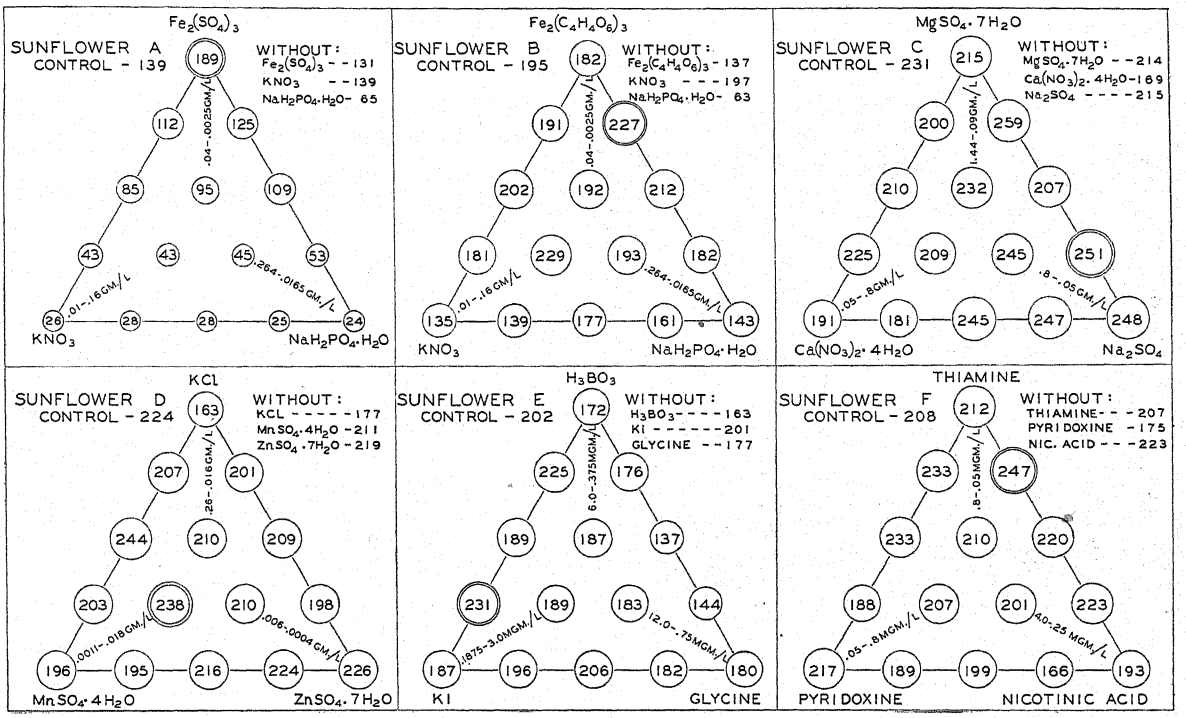


Fig. 2. Effects on the weight in milligrams of excised sunflower tissue cultures grown *in vitro* on different concentrations of mineral salts, glycine, and vitamins. The same method for preparation of the media was used as described in the text and figure 1. Each number represents the average wet weight of 72 tissue cultures run 24 at a time in three separate trials made at different times.

but the exact readings are omitted here because of their volume. The acidities of all media in figures 2 and 3 were within a favorable range for the tissue cultures except in certain media in figures 2, A and 3, A. The influence of unfavorable acidities in the latter cases is considered below.

The results of these experiments were analyzed statistically. The actual F values for each triangular system with sunflower tissue cultures in figure 2, A, B, C, D, E, and F were, respectively, 12.84**, 2.37**, 1.64*, 7.24**, 1.16, and 2.51**. The F values for the tobacco tissue cultures in figure 3 in the same order were, respectively, 5.95**, 13.22**, 2.77**, 1.46, 1.95*, and 1.82*. Significance at the 1 per cent level and at the 5 per cent level, respectively, is indicated by ** and *.

Ferric sulfate inhibited growth of sunflower tissue cultures at concentrations of 0.02 and 0.04 g. per liter and of tobacco cultures at 0.04 g. per liter. Such inhibition was not observed with any of the other salts. This effect of ferric sulfate seemed to be correlated in part with an increased acidity. Thus, with sunflower cultures the media with 0.02 and 0.04 g. per liter, respectively, of ferric sulfate had final pH values ranging from 4.6 to 4.9 and from 3.7 to 4.3. With sunflower tissue there were corresponding increases in pH of the culture media with ferric sulfate concentrations, respectively, of 0.0025, 0.005, 0.01, 0.02, and 0.04 g. per liter. With tobacco tissue, likewise, media with 0.04 g. per liter of ferric sulfate had final acidities ranging from pH 3.7 to 4.2 in one experiment while in two other experiments the pH was within a favorable range. The effect of acidity has been reported already (Hildebrandt, Riker, and Duggar, 1945).

In general the tissues withstood a wide range of concentrations of the various constituents of the basal medium. By varying the concentrations of the constituents of the basal medium plus ferric tartrate, pyridoxine, and nicotinic acid, 108 different media were obtained and were employed with more than 8000 cultures of each of the two species (fig. 2 and 3). For best growth of tobacco cultures, the concentrations of potassium nitrate, potassium chloride, manganese sulfate, glycine, thiamine, and sucrose remained unchanged; while for sunflower cultures, manganese sulfate, thiamine, and sucrose remained unchanged from the concentrations of the basal medium.

Maximum growth appeared when most of the nutrient concentrations were increased. However, for tobacco cultures the concentrations of magnesium sulfate and boric acid were reduced, and for sunflower cultures the concentrations of sodium sulfate and potassium iodide were reduced. Ferric tartrate was a better source of iron for both species than ferric sulfate, which may have been due in certain cases to an unfavorable pH when the latter source of iron was used. A beneficial effect of pyridoxine for sunflower tissue cultures was suggested.

trol cultures, are presented in table 2. Good growth of cultures on media without potassium nitrate does not mean that no nitrate was present because nitrate was added to these cultures as the calcium salt. In a similar manner sulfate was added as iron, magnesium, sodium, and zinc sulfates.

When the data from the triangular 3-salt series and those from a sucrose series (Hildebrandt, Riker, and Duggar, 1945) were examined together, twelve different formulae for media appeared worth trying, were prepared, and were used for cultures of

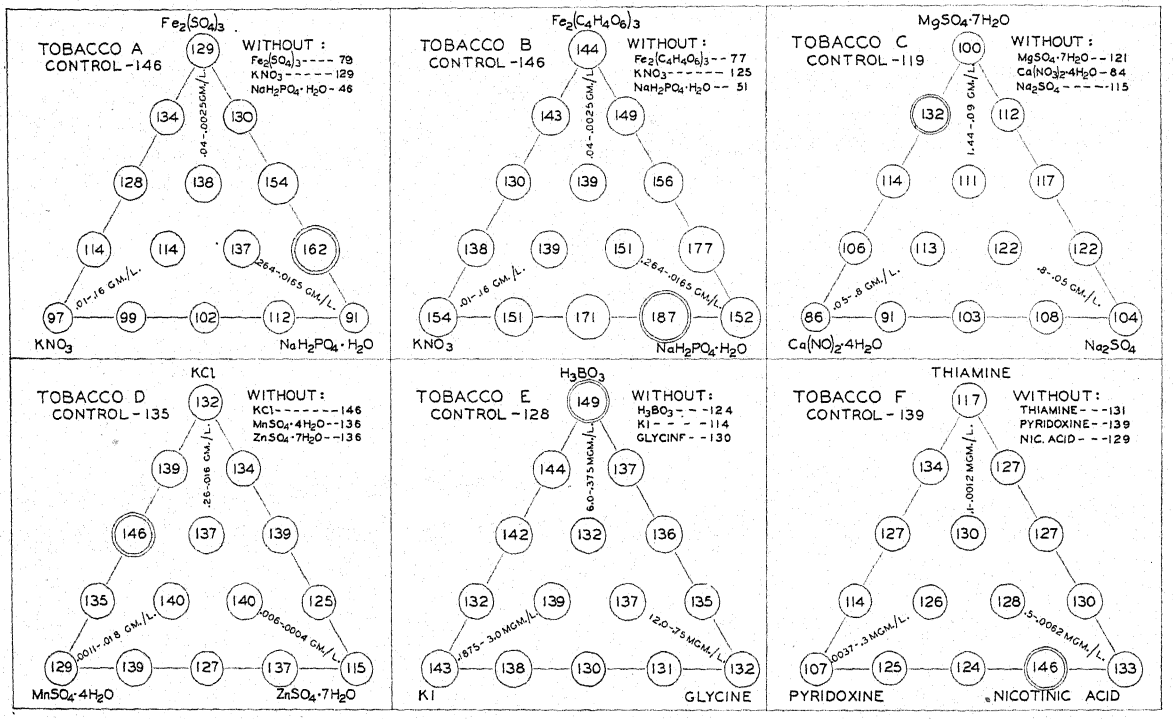


Fig. 3. Same as figure 2 except (1) tobacco tissue and (2) the concentrations of the three vitamins in triangle F were tripled in successive rows. Each number represents the average wet weight of 60 to 84 tissues as explained in the text.

From the data in figures 2 and 3, it may be seen that the best concentrations of each 3-salt series resulted in the percentage increase in growth over the controls, as illustrated in table 1.

The omission of any one nutrient, as indicated at the top of each triangle (fig. 2 and 3), did not mean necessarily that those particular ions were lacking completely, as explained earlier. They were included to see what effect their omission would have on growth in the first passage of the cultures. A better understanding of the effect of the deficiency of any one salt would be had by carrying tissue through several successive passages on media deficient in each salt under test. Thus, a medium without one salt means that it probably had unknown, but very small, concentrations of the ions involved.

The average wet weights of tissues (fig. 2 and 3) grown through one passage on media where one salt was omitted from the otherwise complete medium, expressed as the percentage of growth of control cultures, are presented in table 2. Good growth of cultures on media without potassium nitrate does not mean that no nitrate was present because nitrate was added to these cultures as the calcium salt. In a similar manner sulfate was added as iron, magnesium, sodium, and zinc sulfates.

When the data from the triangular 3-salt series and those from a sucrose series (Hildebrandt, Riker, and Duggar, 1945) were examined together, twelve different formulae for media appeared worth trying, were prepared, and were used for cultures of both tissues. These media had the following general compositions:

1. Basal medium (exact formula given later).
2. Medium 1 plus pyridoxine and nicotinic acid at concentrations, respectively, of 0.1 and 0.5 mg. per liter.
3. Medium 1 with ferric tartrate as the iron source at a concentration of 0.0025 g. per liter.
4. Medium 2 with ferric tartrate as the iron source at a concentration of 0.0025 g. per liter.
5. Improved concentrations of salts and vitamins as determined by the triangular systems (fig. 2 and 3) with ferric sulfate as the iron source and 2 per cent sucrose.
6. Medium 5 except with ferric tartrate as the iron source and the correspondingly better concentrations of potassium nitrate and sodium dihydrogen phosphate (fig. 2, B and 3, B).
7. Medium 1, except 1 per cent sucrose.
8. Medium 3, except 1 per cent sucrose.
9. Medium 1, without thiamine and glycine.
10. Medium 3, without thiamine and glycine.

TABLE 1. *The average increase in wet weights in per cent over controls obtained by the improved concentrations of the 3-salt series illustrated in figures 2 and 3.*

Three-salt series	Increase in average wet weight over controls	
	Tobacco tissue cultures	Sunflower tissue cultures
	per cent	per cent
(A) $Fe_2(SO_4)_3 \cdot 6H_2O$ KNO_3 $NaH_2PO_4 \cdot H_2O$	11	36
(B) $Fe_2(C_4H_4O_6)_3$ KNO_3 $NaH_2PO_4 \cdot H_2O$	28	17
(C) $MgSO_4 \cdot 7H_2O$ $Ca(NO_3)_2 \cdot 4H_2O$ Na_2SO_4	11	9
(D) KCl $MnSO_4 \cdot 4H_2O$ $ZnSO_4 \cdot 7H_2O$	8	6
(E) H_3BO_3 KI Glycine	16	14
(F) Thiamine Pyridoxine Nicotinic acid	5	19

11. Medium 5, except 1 per cent sucrose.
12. Medium 6, except 1 per cent sucrose.

The exact formulae are omitted because of their length and because the essential figures are all given. The experiments were carried out at two different times with 80 tissue cultures on each medium for a total of 160 cultures. Results from these twelve media corroborated results obtained earlier in the 3-salt systems. The average wet weights in milligrams of 160 cultures of each species for each of the twelve media are presented in table 3.

For sunflower and tobacco cultures the best growth was on medium 6 and good growth appeared on media 5, 11, and 12. The addition of pyridoxine and nicotinic acid (media 2 and 4) resulted in slightly increased growth except with medium 2 and tobacco tissue. However, when glycine and thiamine were omitted (media 9 and 10), the growth of sunflower tissue was somewhat larger and of tobacco tissue smaller than the controls. Thiamine and glycine thus seemed beneficial for tobacco tissue, but, since the L. S. D. values for these media (media 2, 4, 11, and 12) were not significant, the necessity of these vitamins and of glycine for these tissues is questionable.

These data also indicated that with the basal medium, slightly better but not significant growth was obtained with 1 per cent sucrose (medium 7). However, with the new concentrations of the various constituents of the medium, better growth occurred with 2 per cent sucrose (media 5 and 6) than with 1 per cent sucrose (media 11 and 12).

TABLE 2. *The wet weights of tobacco and sunflower tissue cultures expressed as the percentage of weight of control cultures on media deficient in one constituent of the basal medium as illustrated in figures 2 and 3.*

Chemicals omitted from basal medium	Average weight of tissues. Controls taken as 100 per cent[a]			
	Tobacco tissue cultures		Sunflower tissue cultures	
	per cent	final pH[b]	per cent	final pH[b]
(A) $Fe_2(SO_4)_3 \cdot 6H_2O$	54*	5.5	94	5.7
KNO_3	88	5.4	100	5.9
$NaH_2PO_4 \cdot H_2O$	32**	5.3	47	5.5
(B) $Fe_2(C_4H_4O_6)_3$	53**	5.4	70	6.0
KNO_3	86	5.5	101	6.1
$NaH_2PO_4 \cdot H_2O$	35**	5.5	32	5.7
(C) $MgSO_4 \cdot 7H_2O$	102	5.6	93	6.2
$Ca(NO_3)_2 \cdot 4H_2O$	71	5.5	73	6.1
Na_2SO_4	97	5.5	93	6.1
(D) KCl	108	5.7	79	6.3
$MnSO_4 \cdot 4H_2O$	101	5.6	94	6.3
$ZnSO_4 \cdot 7H_2O$	101	5.7	98	6.3
(E) H_3BO_3	97	5.6	81	6.0
KI	89	5.6	100	5.9
Glycine	102	5.6	88	6.0
(F) Thiamine	94	5.3	100	5.6
Pyridoxine	100	5.3	84	5.7
Nicotinic acid	93	5.3	104	5.7
Sucrose[c]	30**	6.1	16**	5.8

[a] Significance at the 5 per cent level denoted by *; at the 1 per cent level by **.
[b] Final pH of medium.
[c] From Hildebrandt, Riker, and Duggar (1945).

Stock cultures of tobacco and sunflower tissue were maintained on media containing improved salt concentrations (Hildebrandt, 1944) for over a year. During this period the sunflower tissue grew vigorously. However, the tobacco tissue, although growing better than on the basal medium, often became brown prematurely. Therefore, further studies carried out with the tobacco cultures used the 3-salt systems listed in figure 3. Comparisons of growth of tobacco cultures on these new concentrations (fig. 3), on older concentrations (Hildebrandt, 1944), and on the basal medium were determined by incubating cultures on five media of the general composition indicated below.

1. Basal medium.
2. Improved concentrations from earlier studies (Hildebrandt, 1944) with ferric sulfate as the iron source.

TABLE 3. *The wet weights in per cent of tobacco and sunflower tissue cultures grown on 12 different media compared with control cultures as 100 per cent.*

Medium	Average weight of tissues. Controls taken as 100 per cent[a]	
	Tobacco tissue cultures	Sunflower tissue cultures
	per cent	per cent
1[b]	100 (121 mg.)	100 (130 mg.)
2	99	135
3	107	110
4	108	144
5	125	256**
6	163**	262**
7	114	111
8	108	112
9	88	119
10	90	130
11	116	197**
12	130	247**

[a] Significance at the 1 per cent level indicated by **. None of the others were significant at the 5 per cent level.
[b] Basal medium.

3. Same as 2, except new salt concentrations (fig. 3).
4. Improved concentrations from earlier studies (Hildebrandt, 1944) with ferric tartrate as the iron source.
5. Same as 4, except new salt concentrations (fig. 3).

Forty tissue pieces were incubated in ten flasks on each of the above media. The experiment was repeated twelve times so that a total of 480 tissue cultures was grown on each medium. Growth on the new improved medium (medium 5 above) was significantly better than that on the previously developed medium (Hildebrandt, 1944). The average wet weights of 480 tissue cultures on media 1, 2, 3, 4, and 5 were, respectively, 66, 76, 164, 154, and 195 mg. The L. S. D. at the 5 per cent level was 21 mg. per tissue culture.

The necessity of certain vitamins, such as pyridoxine and nicotinic acid for tobacco tissue and of thiamine and nicotinic acid for sunflower tissue is questionable. Thiamine was retained in the medium for sunflower tissue, while pyridoxine was added. The value of glycine for sunflower cultures is uncertain since a significant F value from the data from figure 2, E was not obtained. The importance of other growth factors and vitamins for these two species was not tested. However, since the tissues grew abundantly, they were apparently synthesizing anything they needed which was not supplied. The composition of the improved media for tobacco and sunflower tissue cultures, as well as the basal medium, is presented in table 4. These studies suggest that these improved media (table 4) could be further simplified.

DISCUSSION.—The present studies have clarified the nutrient balance for growth *in vitro* of sunflower and tobacco tissue cultures. The data have provided a background for a better understanding of results in earlier studies with crown-gall bacterial metabolites and crown-gall tissue extracts.

In general the tissues grew on a wide range of concentrations of the salts tested, but best growth appeared when the concentrations of various items among the nutrients were increased over those of the basal medium. For example, excellent growth of tobacco cultures occurred when the concentrations were increased of sodium sulfate, calcium nitrate, sodium dihydrogen phosphate, iron, zinc sulfate, and potassium iodide. Similarly, with sunflower cultures the concentrations were increased of calcium nitrate, magnesium sulfate, potassium nitrate, potassium chloride, sodium dihydrogen phosphate, iron, zinc sulfate, boric acid, and glycine.

The importance of certain items was especially apparent. With sunflower cultures the omission of sucrose and sodium dihydrogen phosphate resulted in poor growth in the first passage. The omission of ferric tartrate, calcium nitrate, potassium chloride, and boric acid also resulted in inferior growth in the first passage. The lack of the other constituents of the basal medium did not result in a rapid failure of growth, which suggested that their need in the medium needs further examination.

In the case of tobacco cultures the omission of sucrose, iron, and sodium dihydrogen phosphate resulted in poor growth in the first passage. Insufficient potassium nitrate, calcium nitrate, and potassium iodide similarly resulted in inferior growth. The omission of other constituents of the basal medium did not result in inferior growth in the first passage, again suggesting the desirability of more information about the function of each of these materials. From these first passage studies with

TABLE 4. *Composition of improved media for growth in vitro of excised tobacco and sunflower tissue cultures.*

Nutrient ingredient	Basal medium[a]	Tobacco tissue medium	Sunflower tissue medium
Na_2SO_4	200.0 mg.	800.0 mg.	100.0 mg.
$Ca(No_3)_2 \cdot 4H_2O$	200.0 mg.	400.0 mg.	800.0 mg.
$MgSO_4 \cdot 7H_2O$	360.0 mg.	180.0 mg.	720.0 mg.
KNO_3	80.0 mg.	80.0 mg.	160.0 mg.
KCl	65.0 mg.	65.0 mg.	130.0 mg.
$NaH_2PO_4 \cdot H_2O$	16.5 mg.	33.0 mg.	132.0 mg.
$Fe_2(C_4H_4O6)_3$	....	40.0 mg.	5.0 mg.
$Fe_2(SO_4)_3 \cdot 6H_2O$	2.5 mg.	....	....
$MnSO_4 \cdot 4H_2O$	4.5 mg.	4.5 mg.	4.5 mg.
$ZnSO_4 \cdot 7H_2O$	1.5 mg.	6.0 mg.	3.0 mg.
H_3BO_3	1.5 mg.	0.375 mg.	3.0 mg.
KI	0.75 mg.	3.0 mg.	0.375 mg.
Glycine	3.0 mg.	3.0 mg.	12.0 mg.
Thiamine	0.1 mg.	0.1 mg.	0.1 mg.
Pyridoxine	....	....	0.8 mg.
Sucrose	20.0 g.	20.0 g.	20.0 g.
Agar	5.0 g.	5.0 g.	5.0 g.
Water	1000.0 ml.	1000.0 ml.	1000.0 ml.

[a] Basal medium. The water of crystallization is not indicated by White (1942). These salts are as used in these experiments.

both species, it appeared that some of the constituents could either be reduced in amount or possibly eliminated, and the formulae thus might be simplified considerably.

These data have provided a means for improved interpretation of the results observed with crown-gall bacterial metabolites and crown-gall tissue extracts (Hildebrandt, Riker, and Duggar, 1946) on growth *in vitro* of tobacco and sunflower tissue cultures. The effects of different salt balances were seen in the results with the 108 different media in the triangular series. The bacterial culture medium (McIntire, Peterson, and Riker, 1942) contained among other things ammonium sulfate, urea, magnesium sulfate, zinc sulfate, and ferric alum at concentrations, respectively, of 1.0, 0.5, 0.02, 0.002, and 0.005 g. per liter. These salts were not removed entirely when the crown-gall bacteria were separated from the medium, and, therefore, they supplemented the salts of the basal tissue culture medium. Also certain growth factors were synthesized by the bacteria. Similarly lyophilized crown-gall bacterial cells, a polysaccharide from cell-free, crown-gall cultures (Hodgson, Riker, and Peterson, 1945), crown-gall tissue extracts, and yeast extract all contained mineral salts along with unknown materials.

The observed (Hildebrandt, Riker, and Duggar, 1946) stimulating action of these various metabolites and extracts may be due to one or more factors. Deficiencies of the basal medium in organic or inorganic nutrients have appeared as a reason for part of the beneficial action of these materials. Yeast is known to be rich in growth factors, and yeast and crown-gall tissue are high in carbohydrate, ash, and protein.

The increased average wet weights of the tissue cultures on the improved medium (medium 6, table 3) over the basal medium (medium 1, table 3) indicated deficiencies in the basal medium as far as these two species were concerned. For example, the average wet weights of tobacco and sunflower cultures, respectively, were 63 and 162 per cent better on the improved medium (medium 6, table 3) than on the control medium. In earlier work (Hildebrandt, Riker, and Duggar, 1946) tobacco and sunflower cultures on the basal medium supplemented with marigold crown-gall tissue extract, which was one of the most stimulating of the tissue extracts tested, grew, respectively, 63 and 215 per cent better than on the control medium. Thus, it appeared that improved salt concentrations could provide a stimulating effect comparable to that of the marigold gall extract on the growth of tobacco cultures and three-quarters of that on sunflower cultures.

SUMMARY

The influence on growth of over 8000, respectively, tobacco and sunflower tissue cultures in the first passage was studied in 108 different media with increasing and decreasing concentrations, respectively, of the constituents of the basal medium, plus ferric tartrate, pyridoxine, and nicotinic acid. In addition the combined good qualities of new media were studied with 2000 and 4500 cultures, respectively, of sunflower and tobacco tissue.

Results indicated that the concentrations of many of the constituents of the basal medium should be increased.

Ferric tartrate was a better source of iron than ferric sulfate.

The omission of sucrose, iron, and sodium dihydrogen phosphate resulted in poor growth of tobacco tissue cultures in the first passage. There was also less growth when potassium nitrate, calcium nitrate, and potassium iodide were omitted.

The omission of sucrose and sodium dihydrogen phosphate resulted in poor growth of sunflower tissue cultures in the first passage. When ferric tartrate, calcium nitrate, potassium chloride, and boric acid were omitted, less growth appeared than on control cultures.

Pyridoxine appeared favorable for growth of sunflower tissue.

The formulae of the improved media for tobacco and sunflower tissue cultures are given.

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LABORATORY STUDIES ON GLYCERIN AS A SUPPLEMENT IN WATER-SOLUBLE HERBICIDAL SPRAYS [1]

Henry Hopp and Paul J. Linder

WATER-SOLUBLE herbicides such as arsenicals and ammonium sulfamate, applied as sprays, have shown considerable promise for eradication of noxious vegetation. However, results with sprays have been highly variable and, due to the high concentrations of herbicide required, relatively expensive. In order to increase the effectiveness of herbicidal sprays, the following laboratory studies were undertaken during the winter season of 1943–44, to determine the factors that promote transfer of sprayed herbicides from the leaves to the base of the stem.

PROCEDURE.—The tests were conducted on pot-grown plants about 6 inches tall which could be easily manipulated so as to expose them to different conditions and treatments. The results given in this report were obtained with *Coleus blumei* Benth. and *Cuphea platycentra* Lem., two common house plants. The two species appeared to react similarly, so that the principles determined in this investigation may be applicable to a number of species.

Two methods of application were used in the study. In the one, referred to as tip-injection, the solution of poison was placed in a small vial which was so suspended that the tip of the plant could be dipped into the liquid. This method, although not a practical field technique, indicated experimentally the action of the poison in penetrating and being conducted downward through the stem when the foliage was in continuous contact with the herbicide solution. In the second technique, referred to as leaf application, the solution was applied with a camel's hair brush to the upper leaves. This method was analogous to spraying of sprouts in the field. The solutions dried on the surface of the leaf within a short time, usually 10 to 15 minutes.

For the leaf application technique, a commercial sodium lauryl sulfate mixture was added to the herbicide solutions at a rate of 0.12 per cent to serve as a wetting agent.

TABLE 1. *Killing effect of dilute poisons applied by tip-injection at low and high atmospheric humidity (Cuphea plants, 6 inches tall; 14 day period; tested in triplicate).*

Concentration of poison	Inches of stem killed at: Low atmospheric humidity	High atmospheric humidity
Ammonium sulfamate 0.03 molar solution	3, 4, 2	0, 0, 0
Sodium arsenate 0.001 molar solution	4, 4, 3	0, 0, 0
Sodium trichloro-phenoxyacetate 0.001 molar solution	2, 2, 2	0, 0, 0

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The injury to the plants was rated according to the following index:

0 = no injury
1 = treated leaves slightly injured
2 = treated leaves killed
3 = treated leaves and the upper part of stem killed
4 = many leaves and the upper part of stem killed
5 = most leaves and approximately ½ of stem killed
6 = most leaves and approximately ¾ of stem killed
7 = entire plant killed.

The above injury index was used primarily with the leaf applications. With tip-injection, it was possible to measure the actual inches of kill-back on the stem that was injected.

RESULTS.—*Experiment 1.*—It is a common observation that air humidity is a major cause of variation in the killing effect of a given chemical spray on a given plant (Robbins, Crafts, and Raynor, 1942). The effect of high and low atmospheric humidity on translocation of herbicide was tested on *Cuphea* in this experiment. The tip-injection method was used in order to equalize the opportunity for absorption of chemical through the leaves. For the high humidity, potted plants were placed over a tray of water under a transparent cover. The humidity under the cover was very close to saturation. The plants at low humidity were placed on the same bench, but were left uncovered so as to be exposed to the normal dry air of the laboratory. The average air temperature was 67° F. and the relative humidity of the laboratory approximately 30 per cent. Both sets of pots were wrapped in rubberized cloth so that water exchange through the pot walls was similar for both humidity conditions.

Table 1 shows the results after 14 days. No further killing effects were noted. At low humidity each of the three poisons caused dying of the stem, whereas at high humidity none of them killed even the leaves that were submerged directly in the solution.

In the test with sodium trichlorophenoxyacetate the plants in high humidity developed a mass of roots all along the length of the stem a few days after the experiment was started. This peculiar result is characteristic of its effect as a root-promoting substance when introduced into plants, and indicates that it entered and was distributed through the plant even though no killing effect resulted.

The experiment showed in general that a water tension resulting from transpiration is probably more effective than a turgid condition in promoting killing by weak solutions of poisons. These results are in accord with field observations reported by Kennedy and Crafts (1927). At high humidity, the weak solutions used did not kill even the immersed leaves. They were apparently not toxic. However, at low humidity these same concentrations caused kill-back along the stem. Apparently, the herbicide

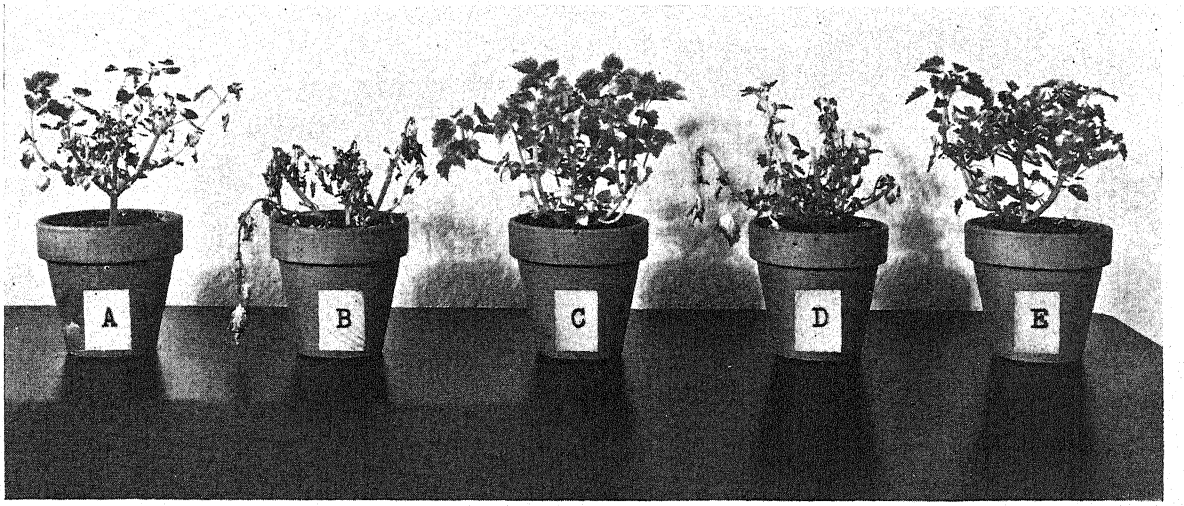


Fig. 1. Effect of sodium arsenate and glycerin applied to the upper leaves of *Coleus* plants. (A) 0.01 M sodium arsenate, (B) same plus 10 per cent glycerin, (C) 0.001 M sodium arsenate, (D) same plus 10 per cent glycerin, (E) 10 per cent glycerin. Photographed on the third day following treatment.

became concentrated within the plant to the point of toxicity.

Experiment 2.—In order to determine the role of absorption in the effectiveness of spray solutions, comparison was made between tip-injection and leaf application of ammonium sulfamate on *Cuphea*. One series was run at low atmospheric moisture and the other at high atmospheric moisture. The results are given in table 2.

Tip-injection at low atmospheric humidity caused considerable kill-back on the stem. Leaf application, even though the solution was approximately three times as strong, caused only slight injury to the leaves under otherwise similar conditions.

At high atmospheric moisture neither method of application caused marked injury. The difference in effect between high and low atmospheric humidity was in accord with the results of experiment 1.

These results indicate that the ineffectiveness of leaf application was due largely to poor absorption of the poison by the leaves rather than to a non-toxic concentration. As a matter of fact, it was evident that at low humidity much of the chemical merely dried on the surface of the leaves and did not get inside the plant. When drying of the solution was eliminated by means of tip-injection, even a lower concentration produced marked killing effects. Under conditions of high atmospheric moisture the solutions were apparently too weak to kill.

Experiment 3.—The above tests suggested the need for a technique that would prevent quick drying of the chemicals on the surface of the leaves, thereby permitting greater absorption into the plant at low atmospheric humidity. The addition of glycerin to the spray solution was found to be an effective agent for this purpose. In one test, using four herbicides, glycerin was added to make 10 per cent of the solution by volume. The herbicides were used in two concentrations, the stronger being ten times the weaker. These concentrations were found in previous tests to be too weak to kill the entire plant without glycerin. The results are shown in table 3. Glycerin alone in water caused slight injury to the leaves, but this effect was minor. Adding the glycerin to the herbicides markedly increased their effectiveness. Figure 1 shows a typical example of the results obtained. This effect of glycerin has recently been shown to hold with 2,4-dichlorophenoxyacetic acid when applied as a dust (Marth, Davis, and Mitchell, 1945).

TABLE 2. *Killing effect of ammonium sulfamate applied by tip-injection and leaf application under low and high atmospheric moisture (Cuphea plants, 6 inches tall; 14 day period; tested in triplicate).*

Method of application	Concentration of ammonium sulfamate	Low atmospheric moisture Kill-back (inches)	High atmospheric moisture Kill-back (inches)
Tip-injection	0.03 molar solution	3, 4, 2	0, 0, 0
		Injury index no.	Injury index no.
Leaf application	0.1 molar solution	1+, 1, 1	1+, 0, 0

TABLE 3. *Surface application of herbicides to upper leaves of Coleus plants with and without the addition of 10 per cent glycerin (14 day period; tested in triplicate).*

Herbicide	Without glycerin (injury index no.)	With 10 per cent glycerin (injury index no.)
Ammonium sulfamate		
0.1 molar solution	2, 2, 2	4, 4, 4
0.01 molar solution	0, 0, 0	2, 2, 2
Sodium arsenate		
0.01 molar solution	5, 5, 5	7, 7, 7
0.001 molar solution	2, 2, 2	5, 5, 5
Sodium trichlorophenoxyacetate		
0.01 molar solution	2, 2, 2	4, 4, 4
0.001 molar solution	2, 2, 2	4, 4, 4
Sodium pentachlorophenoxyacetate		
0.01 molar solution	5, 4, 5	6, 6, 5
0.001 molar solution	2, 2, 2	3, 3, 3
Check		
Water	0, 0, 0	2, 2, 2

Experiment 4.—This experiment was set up to determine the minimum concentration of glycerin required to give the favorable response obtained in the previous experiment. The herbicide used was 0.01 molar sodium arsenate solution. Glycerin was added to make 10, 5, 2.5 and 1 per cent of the solutions by volume. *Coleus* was the test plant. The addition of 2.5 per cent glycerin gave as much kill as 10 per cent glycerin, while 1 per cent glycerin gave somewhat poorer results but still better than that obtained without glycerin.

SUMMARY

The herbicidal action of sodium arsenate, ammonium sulfamate, sodium trichlorophenoxyacetate and sodium pentachlorophenoxyacetate was tested at high and low atmospheric humidity. *Coleus* and *Cuphea* were used as test plants. Results indicated that a high water tension inside the plant resulting from low humidity promoted the effectiveness of herbicides. However, at low humidity the herbicides dried rapidly on the surface of the leaves and absorption was decreased. The addition of glycerin to the solution increased the effectiveness of the herbicide at low humidity. Glycerin probably acted by keeping the poisons in solution for absorption into the leaves over an extended period of time. The addition of this supplementary solvent reduced the amount of herbicide required for killing over that required in a simple water solution.

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THE INFLUENCE OF AUXIN ON RESPIRATION OF THE AVENA COLEOPTILE [1]

J. Berger, Patricia Smith, and G. S. Avery, Jr.

VARIOUS GROSS effects of auxins on plant growth are easily demonstrated and are well known. The exact mechanism, however, by which these changes are initiated still is obscure. A close connection between growth and respiration is almost axiomatic, and Bonner in 1936 demonstrated that such a close relationship existed in the Avena coleoptile. This logically suggests a study of the effect of auxin on respiration, as a means of elucidating the fundamental mechanism of auxin action. A number of approaches to such a study might be made, for example:

1. to demonstrate an "in vivo" auxin stimulation of the total respiration.

2. to demonstrate an "in vivo" auxin stimulation of a specific portion of the respiratory cycle.

3. to conclusively demonstrate, "in vitro," auxin stimulation of a single chemical reaction.

Previous work in this laboratory has employed the latter two approaches. It was found that two specific cell-free enzyme systems of the Avena coleoptile (alcohol and malic dehydrogenases) were stimulated by pre-treatment of intact tissue with auxin (Berger and Avery, 1943c). However, attempts to show an "in vitro" auxin stimulation of dehydrogenase activity, using indoleacetic acid as the auxin, were unsuccessful (Berger and Avery, 1943b). The first approach was used by Commoner and Thimann (1941), who showed that under certain conditions, indoleacetic acid stimulates the oxygen uptake of living segments of Avena coleoptile tissue. Inasmuch as we were able to duplicate this result, and no other confirmation of their report has appeared, it seems desirable to publish the results of the present work, which has had to be discontinued. Commoner and Thimann also reported that the auxin stimulation was greatly magnified by the presence of malate (or fumarate), a result which we have been unable to duplicate.

MATERIALS AND METHODS.—Hulled seeds of *Avena sativa,* variety Victory, were soaked in distilled water for 2 hours, then set out on moist filter paper in 14 cm. Petri dishes, and grown in the dark, with periodic red light, at 25°C. and 80–90 per cent relative humidity for 55–57 hours. After removal of the coleoptile tip and its inclosed foliage leaf, the coleoptile cylinders were cut into three segments, each about 4 mm. long, and soaked for 14–16 hours in various solutions, in the dark at 25°C. Thirty segments from ten coleoptiles were then placed in fresh solutions in each respirometer flask. Respiration was measured in Warburg manometers, shaken at a constant rate of 60 times per minute. The systems were first allowed to equilibrate for 30–60 minutes in the respirometer water-bath, which was maintained at 30°C. in diffuse daylight. Exceptions to this general procedure will be noted later where they are made.

The final volume of reaction-mixture in each flask was 2.5 ml. and the final concentrations of reagents, unless otherwise specified, were: potassium *l*-malate, pH 7.0, 0.001 molar; indoleacetic acid (IAA), 10 mg./liter or 5.7×10^{-5} molar; and sucrose 1 per cent. These are the same concentrations employed by Commoner and Thimann. The center well of each flask contained 0.1 ml. of 10 per cent potassium hydroxide. In general, experiments were set up in such a way as to duplicate as nearly as possible the conditions used by Commoner and Thimann.

EXPERIMENTAL RESULTS.—*Influence of auxin and malate on respiration.*—To test for possible respiratory stimulation by added indoleacetic acid or malate, segments were "starved," *i.e.,* depleted of excess substrate and auxin by soaking them for 18 hours in distilled water. The respiratory responses of these segments in fresh solutions are shown in table 1 and figure 1. It may be seen from the results of all three experiments of table 1 that addition of malate to the control causes only a very slight increase in total oxygen uptake, but that the addition of auxin to the control gives increases of 28–40 per cent. The increase in respiration occurs almost immediately (fig. 1) and is stable for at least three hours. On the other hand, separate experiments show that the addition of auxin to segments respiring in *water* (no sucrose) generally causes *no* significant change in the rate of respiration (confirming Com-

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This paper reports work carried out at Connecticut College.

TABLE 1. *Auxin stimulation of respiration in segments of Avena coleoptile tissue, previously starved by soaking in water. The auxin used throughout is indoleacetic acid (IAA).*

Reaction mixture	cmm. oxygen uptake of segments of tissue pre-soaked 18 hours in water			Average per cent stimulation over control
	Expt. 1 165 minutes	Expt. 2 210 minutes	Expt. 3 190 minutes	
Sucrose control	98	74	120	..
Sucrose + malate	108	85	126	9
Sucrose + auxin	126	104	167	35
Sucrose + auxin + malate	127	110	167	39

moner and Thimann). The further addition of malate to sucrose and auxin does not significantly increase the oxygen uptake over the sucrose-auxin combination. Thus the data confirm Commoner and Thimann's findings as to stimulation of oxygen uptake by auxin, but fail to demonstrate any role of malate.

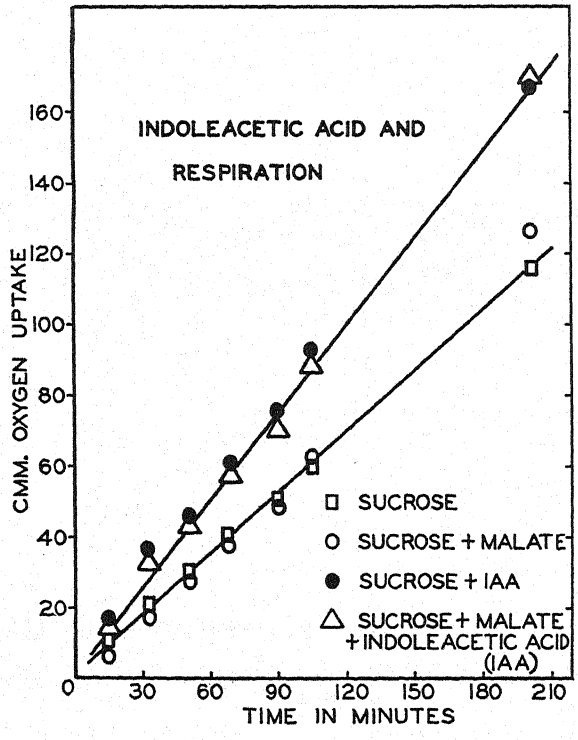


Fig. 1. Oxygen uptake of coleoptile tissue, first starved by soaking in distilled water for 18 hours, then put into solutions containing sucrose, etc., as indicated in the graph.

To further test for possible malate participation in auxin activity, segments were depleted of any naturally occurring auxin by soaking 14 hours in sucrose solution, or depleted of malate by soaking in sucrose-auxin solution. The segments were then placed in fresh solutions and oxygen uptake measured. Results are shown in table 2. It is clear that malate does not stimulate any combination tested. That the segments were capable of increased respiration is shown by the 18–33 per cent stimulation caused by the addition of auxin to the reaction mixture. According to Commoner and Thimann the 4-carbon acids increase the respiration only in the presence of auxin, hence one would have expected to get a stimulation of respiration upon addition of malate to segments soaked in sucrose + auxin, but not with segments soaked in sucrose alone.

In another experiment segments were first soaked in malate solution or in water, for 14 hours, and then put in fresh solutions for respiration measurements, with the results shown in table 3. Again it may be seen that the addition of auxin to sucrose increased oxygen uptake by 21–53 per cent (an average of 35 per cent) over sucrose alone, but the further addition of malate (expts. 2 and 3) caused no greater increase.

Effect of malate concentration.—Since no marked stimulation could be obtained with 0.001 molar malate, a series of concentrations was tested. Segments were soaked in water or in auxin solution for 14 hours. The oxygen uptake of such segments was then measured in the presence of 0.0001, 0.0005, 0.001, 0.005, 0.01 and 0.05 molar malate plus sucrose both in the presence and absence of added auxin. In all experiments, the total uptake at any given time increased slightly with increasing malate concentration up to 0.01 to 0.05 molar as shown in figure 2, *i.e.*, the malate apparently acts as a substrate, and not as a catalyst. If the action of malate were catalytic, one would expect to obtain an accelerating effect at *low* concentrations, such as Commoner and Thimann presumably found at 0.001 M. But we find that oxygen uptakes increase uniformly with increasing malate concentration up to 0.05 M; this is commonly accepted to be the range of *substrate* concentration, not catalyst concentration.

Effect of auxin concentration.—Segments from 54 hour old coleoptiles were soaked in sucrose for 15 hours to deplete the tissues of naturally occurring auxin; respiration measurements were then made in a solution of sucrose and the following auxin concentrations: 0.01, 0.1, 1.0 and 10.0 mg/liter of indoleacetic acid. All concentrations of auxin tested increased the oxygen uptake over the

TABLE 2. *The influence of added auxin and malate on respiration in segments of Avena coleoptile tissue, previously depleted by soaking in sucrose or sucrose-auxin solutions.*

Reaction mixture	cmm. oxygen uptake of 30 segments				Average per cent stimulation over control
	Expt. 1, 200 minutes		Expt. 2, 215 minutes		
	Pre-soaked in sucrose 14 hours	Pre-soaked in sucrose—IAA 14 hours	Pre-soaked in sucrose 14 hours	Pre-soaked in sucrose—IAA 14 hours	
Sucrose, control	141	164	107	129	..
Sucrose + malate	141	166	113	131	2
Sucrose + malate + auxin	171	216	133	174	28

control (which had no indoleacetic acid added), with an apparent optimum of 1 to 10 mg/l.

Discussion.—In a comparison of the results obtained here and by Commoner and Thimann, an outstanding point is the agreement on auxin stimulation of oxygen uptake by coleoptile tissue. On the other hand, no evidence was found here for the participation of malate as a catalyst in the mechanism of auxin action, as reported by Commoner and Thimann. This lack of confirmation is difficult to explain, although certain differences in technique might possibly be the explanation. Thus, Commoner and Thimann used 76 hour old coleoptiles, and measured the respiration of the segments at 24°C. in the dark. In our study a temperature of 30°C. has been used in order to increase the total uptake; diffuse daylight was used for convenience and in the absence of any evidence for the need of darkness; finally, 55–57 hour old coleoptiles were selected because the variety Victory has been shown to be most sensitive to auxin between the age of 55–74 hours. This is the period when the coleoptile is used for hormone assay. Experiments were carried out to determine whether differences in respiratory stimulation could be attributed to age of coleoptile tissue. It was found that the addition of malate to previously starved segments (pre-soaked in water 12 hours) respiring in sucrose-auxin solution does *not* give a significant stimulation of oxygen uptake, whether with 52 or 76 hour old tissue. Moreover, the per cent stimulation over a malate control on addition of auxin and sucrose is the same with both ages of tissue.

The effects of temperature on respiration and auxin-malate relations were not investigated in this study, but at least one variety of Avena has its optimum growth at 30°C. (Newcombe, 1923).

Three experiments carried out in darkness for the first hour, and in light for the next hour and one-half also failed to give any evidence of malate stimulation of respiration in the presence of auxin.

However, it is obvious from the data that even with the differences in technique, it was possible to demonstrate stimulation by auxin to as great or greater an extent than that reported by Commoner and Thimann.

Commoner and Thimann showed (their fig. 10a and fig. 10b) that with increasing concentrations of fumarate up to 0.005 molar, there was a marked increase in respiration rate that was *not* accompanied by an increase in growth. On the other hand, malate behaved exactly the opposite: increasing concentrations of malate up to 0.01 molar *did* produce a marked increase in growth but not in respi-

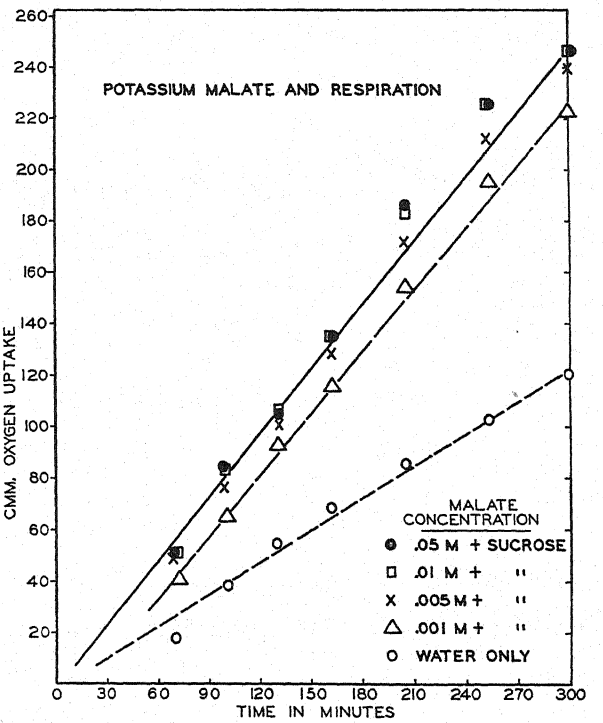


Fig. 2. Effect of malate concentration on oxygen uptake. Coleoptile segments were pre-soaked 14 hours in a 10 mg./liter solution of indoleacetic acid, then put into fresh solutions of water, or of sucrose plus a range of concentrations of malate.

Table 3. *Effect of added auxin and malate on respiration in segments of Avena coleoptile tissue previously depleted by soaking in water or malate solution.*

Reaction mixture	cmm. oxygen uptake of 30 segments: Expt. 1, readings after 180 minutes — Pre-soaked in water 14 hours	Expt. 1, readings after 180 minutes — Pre-soaked in malate 14 hours	Expt. 2, segments pre-soaked in malate, 14 hrs.; readings after 240 minutes	Expt. 3, segments pre-soaked in malate, 14 hrs.; readings after 180 minutes	Average per cent stimulation over sucrose control
Water	61	65	74	64	..
Sucrose, control	104	101	130	101	..
Sucrose + malate	...	...	144	119	14
Sucrose + auxin	126	128	178	155	45[a]
Sucrose + auxin + malate	...	...	180	147	42

[a] Average of experiments 2 and 3.

ration. Since fumarate is rapidly converted to malate according to the four-carbon acid system (and indeed by *Avena* coleoptile enzymes [Berger and Avery, 1943a]), these two compounds should behave *similarly*. The substrate role that Commoner and Thimann show fumarate to play at 0.005 molar concentration seems to be the part played even by 0.001 molar malate in the experiments of the present investigation. Previous work with isolated malic dehydrogenase from the *Avena* coleoptile shows that even 0.0005 molar malate is enough to act as a substrate, and not necessarily as a catalyst (Berger and Avery, 1943a).

SUMMARY

The respiration of intact Avena coleoptile tissue in 1 per cent sucrose solution is stimulated by about 35 per cent in the presence of 10 mg./l. of indoleacetic acid.

Tissues starved of excess carbohydrate substrate, and depleted in auxin content, give a stimulated respiratory rate in sucrose solution when indoleacetic acid alone is added. Simultaneous addition of the four-carbon acid, malic, does not catalytically increase the oxygen uptake.

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OSMOTIC VALUES OF BRYOPHYTES AND PROBLEMS PRESENTED BY REFRACTORY TYPES [1]

Paul M. Patterson

The relative dependence of certain bryophytes and bryophytic societies upon moisture was determined by Patterson (1940a and 1940b). There followed an investigation of the pectin content, absorption of moisture from air containing different levels of humidity, and the respiration rates of a series of bryophytes (Patterson, 1943). In the latter work, the bryophytes studied were typical representatives of xeric, mesic, and hydric habitats. There was no correlation between the character under consideration and the habitat preferences of the bryophytes.

The present work was a continuation of the latter study in which another property, the osmotic value of the cell sap of bryophytes, was investigated. An endeavor was made to determine whether (1) significant osmotic differences exist in the cell sap of bryophytes collected from a wide range of ecological habitats, and (2) whether the osmotic differences of the various species are correlated with the amount of available moisture in the habitat.

Renner (1933) has reported differences in the osmotic pressure of the cell sap of certain epiphytic lichens, algae, bryophytes, and ferns in Java and adjacent areas. He used the plasmolytic method in determining osmotic values. The intervals between the concentrations of his series of testing solutions were large. Most of the epiphytic bryophytes Renner tested were plasmolyzed by a solution of a single concentration. This may have been due either to the physiological uniformity of the ecological group of bryophytes, or to the wide differences in the concentrations of the testing solutions he used.

Materials and methods. — The plasmolytic method was used here for testing the osmotic concentration of the cell sap. Although other solutions were occasionally employed, as noted below, in most instances a KCl solution was used to which was added sufficient $CaCl_2$ to make the ratio of K^+ and Ca^{++} 49:1. The antagonistic Ca^{++} was added to reduce the possible toxicity of a solution containing K^+ as the only cation. The solutions were made up in 0.1 N intervals. It was felt that closer intervals of concentrations such as were used by Becker (1931) would be of no special value in this work.

Although there are inherent inaccuracies in the plasmolytic method, this technique is the only practical means available for determining the osmotic values of bryophyte cells. These inaccuracies have been discussed recently by Currier (1944).

The leaves of mosses and leafy hepatics, and the superficial and marginal cells of the thalloid hepatics were the materials used to determine the osmotic values. Only mature, healthy tissues from the apical ends of the plants were selected for testing. These were rendered turgid by soaking in

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This work was done in part with the assistance of two General Education Board Research Fellowships at the Mountain Lake Biological Station of the University of Virginia during the summers of 1944 and 1945. An abstract of a part of this work was published in the American Journal of Botany 31 (8): 4s. 1944 under the title, "Osmotic values of bryophytes and problems in permeability."

water, or collected wet and kept in this condition when they were to be used as test materials.

Precautions were taken to prevent significant changes in the concentrations of the testing solutions during the preliminary immersion of the bryophytes and while they were under observation with the microscope. Thirty to forty minutes or less was found to be sufficient time for the materials to reach approximate aqueous equilibrium with the testing solutions. During this interval, several observations were made on the tissues that were in the salt concentrations which approximated that of the cell sap.

When leaf cells were obscured by the presence of large or numerous papillae, observations were confined to the basal area of the leaves where the papillae were low or absent. Thick, opaque leaves such as those of *Polytrichum,* were split horizontally just before microscopic examination.

The determination of the point of incipient plasmolysis varied with the species of bryophyte under observation. In some instances, when testing with the ascending series of tenth-normal concentrations, plasmolysis occurred in the majority of cells over the entire leaf lamina with the first solution that was hypertonic. When this plasmolysis was very slight, the concentration producing it was taken as the equivalent to that of the cell sap; when the plasmolysis was conspicuous, the osmotic equivalent was recorded at a figure 0.05 N below this concentration. It is presumed that errors thus introduced would cancel out when computing the average of a number of determinations. In other bryophytes, plasmolysis occurred in the basal quarter of the leaf at first, and a stronger concentration was required to induce plasmolysis in the distal leaf cells. In such cases the osmotic equivalent of the leaf cells was recorded as the average of the concentrations which plasmolyzed the base and tip of the leaves, if this difference was not greater than 0.1 N. When greater, the concentration which plasmolyzed the basal part of the leaves was recorded. A type of plasmolytic behavior similar to that just described was observed in *Catharinea* (*Atrichum*) *undulatum* by Lambrecht (1929). In a few other bryophytes, the cells that plasmolyzed first were few in number and scattered at random over the whole area of the leaves and a more concentrated solution was required to plasmolyze all of the protoplasts. The osmotic value of such tissues was determined in the same manner as that just described.

THE OSMOTIC VALUES OF BRYOPHYTES.—The bryophytes tested for their osmotic values are grouped according to their apparent dependence upon moisture. Those included in the table below under *xeric species* grow on tree trunks, boulders, and cliffs that become dry. Under *mesic species* are placed those growing typically on the bases of trees, logs, humus and soil. The *hydric species* include bryophytes actually submerged or constantly wet with water. An attempt was made to collect bryophytes for testing from typical habitats. In a few instances, where these places were not quite typical, the bryophytes were put in the groups which the particular collection sites indicated. For instance, *Dumortiera hirsuta* would be grouped normally with the mesic instead of the hydric species except for the fact that it grew in constantly running or dripping water at the station from which it was secured.

Both temperature and light intensities influence the osmotic concentration of the cell sap. All of the tests for osmotic values were made during the spring and summer seasons. Light would be expected to influence the quantity of osmotically active substances in moist bryophytes as a result of photosynthesis. Copeland (1896) reported that the normal variation in osmotic values between the evening of a sunny day and the following morning in *Funaria* was equivalent to 0.5 per cent KNO_3 (approximately 0.05 N). When *Funaria* was kept in the dark, its osmotic value had declined by 2.5 per cent KNO_3 (about 0.25 N), by the end of five days. Lambrecht (1929) has also demonstrated that light has an effect upon the osmotic pressure of the leaf cells of *Catharinea* (*Atrichum*). The writer has observed the effect of light in several instances. Two collections of *Fontinalis dalecarlica* were taken from the same stream at the same time on the afternoon of a clear day and tested promptly after collection. One sample was growing at a road crossing where it was exposed to sunshine for the greater part of the day, while the other occurred in the dense shade of a *Rhododendron maximum* thicket a few feet distant. The plant in the sun showed 0.1 N KCl greater osmotic value than the one in the shade. *Marsupella sphacelata* was growing in a moist condition in the sunlight on rocks in the same stream with *Fontinalis*. When collected and tested on the afternoon of a sunny day it had an osmotic value equivalent to 0.25 N KCl higher than a similar collection taken at the same time and kept moist in the dark for 65 hours. *Mnium affine* collected early in the morning from a deeply shaded habitat gained the equivalent of 0.1 N KCl in osmotic value when exposed for 5½ hours to bright daylight in a moist chamber. *Scapania nemorosa* var. *alata* (Kaal.) K. Muell. showed no change in osmotic value after 65 hours in the dark. In routine testing, variations in light as extreme as those indicated above were excluded. The bryophytes were collected from their normal habitats and tested the same or the following day. With the concentration intervals of the testing solutions used, holding the bryophytes overnight introduced no significant changes in the osmotic values of cell sap. This was indicated by tests made with a number of species, including *Bryum capillare, Dicranum scoparium, Plagiochila asplenioides,* and *Tortula pagorum.* It is thus considered that the differential effect of light upon osmotic concentrations of the cell sap of bryophytes as a result of the testing procedures followed here was insignificant. Further, such insignificant variations in osmotic values as might have resulted would

TABLE 1. *Osmotic values of bryophytes. The bryophytes are grouped according to their requirements for moisture. The osmotic equivalent for the cell sap is indicated for each species in terms of KCl normality. The starred (*) species are thalloid hepatics and they are excluded in calculating the means for each group.*

	Osmotic values in KCl normality	
Xeric species		
Bazzania denudata	0.50	
Dicranum fulvum var. *viride*	0.40	
Fabronia ciliaris	0.45	
Frullania riparia	0.55	
Frullania squarrosa	0.60	
Homalia sharpii	0.70	
Homalia jamesii	0.55	
Hyophila tortula	0.50	
**Metzgeria crassipilis*	0.40	
Microlejeunea laetevirens	0.50	
Neckera pennata	0.55	
Plagiochila asplenioides	0.45	
Plagiochila ludoviciana	0.50	
Porella platyphylloidea	0.65	
Sphenolobus minutus	0.45	
Tortula caroliniana	0.30	
Tortula fragilis	0.45	
Tortula pagorum	0.45	
Tortula papillosa	0.50	
Arithmetical mean of the xeric species		0.502
Mesic species		
Anomodon attenuatus	0.55	
Atrichum angustatum	0.45	
Atrichum crispum	0.30	
Atrichum undulatum	0.35	
Bazzania trilobata	0.60	
Blepharostoma trichophyllum	0.45	
Bryum argenteum	0.40	
Bryum capillare	0.35	
Cephalozia bicuspidata	0.35	
Ceratodon purpureus	0.40	
**Conocephalum conicum*	0.20	
Dicranum flagellare	0.40	
Dicranum scoparium	0.45	
Dicranum spurium	0.45	
Diphyscium foliosum	0.55	
Diplophyllum apiculatum	0.45	
Entodon cladorrhizans	0.55	
**Fossombronia wondraczekii*	0.25	
Hookeria acutifolia	0.25	
Lepidozia reptans	0.50	
Leucobryum glaucum	0.30	
Lophocolea heterophylla	0.40	
**Marchantia polymorpha*	0.20	
Microlepidozia sylvatica	0.55	
Mnium affine	0.25	
Mnium marginatum	0.45	
Mnium punctatum	0.35	
**Notothylas orbicularis*	0.20	
Nowellia curvifolia	0.30	
**Pellia epiphylla*	0.15	
**Pellia neesiana*	0.15	
Plagiothecium sylvaticum	0.45	
Plectocolea crenulata	0.35	
Plectocolea crenuliformis	0.35	
Pogonatum pennsylvanicum	0.40	
Polytrichum ohioense	0.35	
**Riccardia multifida*	0.15	
Rhodobryum roseum	0.20	
Scapania nemorosa	0.45	
Tetraphis pellucida	0.40	
Thelia asprella	0.50	
Thuidium delicatulum	0.35	
Arithmetical mean of mesic species		0.405
Hydric species		
Aulacomnium palustre	0.55	
Drepanocladus aduncus var. *polycarpus*	0.50	
**Dumortiera hirsuta*	0.30	
Eurhynchium rusciforme	0.55	
Fissidens grandifrons	0.55	
Fontinalis dalecarlica	0.35	
Fontinalis novae-angliae	0.35	
Hygroamblystegium irriguum	0.40	
Jubula pennsylvanica	0.40	
Leptodictyum riparium	0.55	
Marsupella sphacelata	0.45	
Mnium punctatum var. *elatum*	0.30	
Nardia geoscyphus	0.35	
Philonotis fontana	0.30	
Porella pinnata	0.55	
Scapania nemorosa var. *alata*	0.45	
Trichocolea tomentella	0.35	
Arithmetical mean of the hydric species		0.434

tend to cancel out when a number of different tests were averaged.

Except where authorities are cited, the names used in this paper for the mosses, for *Sphagnum*, and the hepatics are those listed by Grout (1940), Andrews (1940), and Evans (1940), respectively.

The arithmetical mean for all of the mosses and leafy hepatics together is 0.438 N KCl; that for the entire list is 0.414. The thalloid hepatics, as a whole, have a low osmotic value (0.222). They have been disregarded in the calculation of the means of the ecological groups because their numerical occurrence is not proportionately distributed in the three categories, and to include them would lower the mean of the mesic species differentially.

With the thalloid hepatics excluded, the *standard error of the difference* between each pair of means was computed. The significance of the difference between these means is indicated in table 2.

The bryophytes are listed according to their degree of dependence upon moisture. There is no posi-

TABLE 2.

Difference between the means of	The probability of deviations as great by chance alone	Statistical significance
Xeric and mesic species	below 0.1%	strong
Xeric and hydric species	3%	weak
Mesic and hydric species	about 23%	none

tive correlation between the osmotic values of the bryophytes studied and their adaptations to moisture, else the hydric group would have a significantly smaller mean osmotic value than the mesic. On the other hand, light intensity differs to a considerable degree in these three average habitats. The xeric species, with several exceptions, were collected from areas where light intensity was rather high (lightly shaded areas). The mesic group, for the most part, were collected from heavily wooded habitats. Statistically the difference between the means of these two groups is highly significant. The hydric species show an increase in the mean osmotic value over the mesic species instead of an expected decrease. Thus the significance between the xeric and hydric means is much smaller. This very fact becomes significant when it is pointed out that all but one of the hydric species with an osmotic value of 0.50 N or greater were collected from ponds, pond shores and streams exposed for all or part of the day to full daylight. Thus it would seem that the increase in the mean of the hydric over the mesic species would be accounted for by the increase of the light intensity for some of the species included. The average osmotic values, then, seem to reflect the different light intensities at these several composite habitats. In general, there seems to be a positive correlation between the habitat and the osmotic value of the bryophyte; and the intensity of light, and not the amount of available moisture, is indicated as a primary factor in the osmotic differences.

SPECIES WITH NORMAL OR ATYPICAL PLASMOLYTIC BEHAVIOR.—Several types of behavior with respect to plasmolyzing agents were encountered. These types have been numbered, and are discussed below.

Type 1.—When other factors remain unchanged, a large number of bryophytes showed the same osmotic value after a prolonged moist period (24 hours or more), that they did when wetted and tested immediately after a period of desiccation. This behavior was the most common and thus seems to be the normal one. These bryophytes are here designated as showing uniform osmotic values. Typical bryophytes behaving in this manner were *Bryum capillare, Metzgeria crassipilis, Plagiochila asplenioides, Scapania nemorosa,* and *Tortula fragilis.*

Type 2.—The bryophytes put in this category showed what is here called an incipient refractory period. These had a somewhat higher osmotic value when tested after a normal period of desiccation than they did after a moist period of 24 hours or more. The figures following the names below indicate the apparent increase in terms of a normal KCl solution: *Atrichum angustatum* (0.30), *Cephalozia bicuspidata* (0.20), *Dicranum flagellare* (0.10), *Fabronia ciliaris* (0.10); *Plagiochila ludoviciana* (0.20), *Polytrichum ohioense* (0.40). Ursprung and Blum (1916) observed a similar behavior in *Funaria.*

Type 3.—The bryophytes placed in this category had a more strongly marked refractory period. When these species were wetted and tested within two hours after a period of desiccation, protoplasts of leaf cells failed to plasmolyze in solutions as high as 2.0 N KCl, or some cells showed a small amount of irregular shrinkage instead of typical plasmolysis. Only three forms are known in this group, *Sphenolobus minutus, Frullania riparia,* and *Porella platyphylloidea.* Although *Porella* is placed here, its behavior varied in different collections and tended to be intermediate between types two and three. *Sphenolobus* is placed here on the basis of a single test. *Frullania* is typical of the group. When kept moist after a period of desiccation, return to normal plasmolytic behavior was gradual over the next 24–48 hours.

Type 4.—This group fails to plasmolyze in a typical manner after desiccation during periods observed for as long as 23 days. This category consists of mosses only. This group is known as the permanently refractory type. Permanency of the refractory nature was assumed for a number of species where no change in character was observed after a week of continuous hydration; for other species, after two days.

In highly hypertonic solutions (*e.g.,* 2 N KCl), some cells may be plasmolyzed in the alar or in several cell layers at the base of the leaf. Only rarely were typically plasmolyzed cells seen in the leaf blade. Sometimes plasmolysis occurred in immature leaves. Further, a slight or marked aberrant shrinkage of the protoplast was often seen in many of the leaf cells which did not look like typical plasmolysis since the visible edge of the protoplast was usually irregular. Often no sign of shrinkage was seen. This is not incipient plasmolysis in the usual sense since large differences in concentration have little effect on the amount of shrinkage.

About forty mosses were observed which showed this property. The following were typical: *Amblystegium serpens, Andreaea rupestris, Hygrohypum eugyrium, Hylocomium brevirostre, Hypnum cristacastrensis, Hypnum imponens, Leucodon julaceus, Mnium hornum, Rhacomitrium aciculare, Sphagnum palustre,* and *Ulota americana.*

Type 5.—This category consists of bryophytes that may plasmolyze readily after a period of desiccation in one sample and fail to do so in another. *Bazzania trilobata, Leucobryum glaucum* and *Fontinalis dalecarlica* showed this behavior. *Fontinalis* grows in running water, and in our streams is wet during the entire summer. It is of special interest because it demonstrates that the refractory character may be acquired in environments where periodic desiccation does not occur during the period of observation.

Several aspects of this refractory problem may now be examined.

1. Failure to plasmolyze after a period of dryness may be normal for the species (*e.g.,* type 3), or may be caused by the death of the cells as a result of drying. It is imperative to recognize moribund and dead cells and to confine all observations to nor-

mal, living tissue; thus care was taken to distinguish between living and moribund or dead cells.

2. In some instances, if the cytoplasm had no typical large central vacuole, plasmolysis might be so slight as to pass unnoticed, particularly in very narrow, elongated cells with small lumina. However, for *Hypnum imponens* and *H. crista-castrensis,* whose leaf cells are among the narrowest of the mosses studied, stained microtome sections showed the typical peripheral cytoplasm surrounding a proportionately large central vacuole.

3. The question may arise as to whether a greater degree of desiccation than is attained by air drying will induce refractoriness in species that otherwise plasmolyze normally when they are wet after a period of dryness (type 1). Drying plants over calcium chloride resulted in the death of the mature leaves only, or all of the leaves, including those of the vegetative buds, in the more mesic species. However, many species survived this greater degree of desiccation but they showed no change in their plasmolytic behavior.

4. It may be presumed that the mosses that fail to plasmolyze at any time (type 4), have an unusually high osmotic concentration of the cell sap, and that the testing solutions were not sufficiently concentrated. While 2 N KCl was used normally to determine the mosses' capacity to be plasmolyzed, a number of species were further tested with a 4 N KCl and also with 50 per cent glycerin (about 6.8 molar solution). Concentrations of glycerin above 60 per cent produced no plasmolysis, but induced the shrivelling effect of progressive desiccation. Sucrose and salts other than KCl were also used in strong concentrations without effecting typical plasmolysis. Most of the experimentation with the latter was done with *Leucodon julaceus.*

5. It may be suggested that adhesion of the cytoplasm to the cell wall might be a factor in preventing plasmolysis, but where this has been measured, as in seed plants, the interference is small. We would therefore not expect it to play a significant role in these plants where strongly hypertonic solutions were used.

6. The viscosity of the cytoplasm, or a change in colloidal phase may be involved where cytoplasm shows a refractory character. If bryophytes from type 1 are sectioned in the fresh, turgid state and are placed in water, the cytoplasm almost always leaves the open cells and becomes finely dispersed in the water during the process of making a slide for observation. In a number of non-plasmolyzable mosses of type 4, sections of leaves in water with one exception showed the cytoplasm oozing out from the cut ends of the cells in vermiform protrusions. There was also more or less dispersion at the ends of the extruded protoplasts. In some instances, pressure had to be applied to force the cytoplasm out of the cells. This is a crude indication that different degrees of viscosity exist. When moist leaves of *Leucodon julaceus* were sectioned and mounted in water, the protoplasts in opened cells were jarred out of the cell walls by tapping the coverslip. This treatment fragmented many protoplasts, but others remained intact and maintained the original shape imposed by the containing cell walls. These protoplasts did not seem to be plastic, but rigid and brittle, which may indicate that the cytoplasm was in a gel state. Fritsch and Haines (1923) state that for several terrestrial algae and for moss protonema, cells surviving after drought "are characterized by a more or less rigid, highly viscous protoplast." They also suggest that "the gel condition of the protoplast with absence of all large vacuoles indicates the possibility of imbibition phenomena being chiefly concerned." It seems possible that mosses with the cytoplasm in a highly viscous or gel state may shrink irregularly when surrounded by highly hypertonic solutions.

7. Bryophytes in the process of drying or hydrating may undergo changes in osmotically active materials within the protoplasts. Northen (1943) working with a species of *Mnium* and another of *Bryum* found that incipient drought brought about a decrease in structural viscosity as attested by centrifuging. He attributed this to protein dissociations. With the use of *Frullania riparia,* the writer verified Northen's observation. Golovina (1942) concluded from his study of seed plants that the velocity of hydrolysis and content of reducing sugars were greater in wilting plants than in control plants. From the work of Golovina and others, Northen suggested that the dissociation of cellular proteins by incipient drought conditioned polysaccharide hydrolysis, along with other changes in cellular materials. The increase due to polysaccharide hydrolysis is probably small and with the testing technique used, this increase has not been observed by plasmolytic tests in bryophytes belonging to type 1. If no other groups were known, type 2 might fit into this interpretation. The other types, obviously, require another explanation.

8. Permeability or impermeability of the plasma membrane may contribute to the refractory character of the bryophytes. To test whether the plasma membrane may be impermeable to water in these types, a number of fat soluble substances such as several of the alcohols (ethyl alcohol, principally, but also methyl and tertiary butyl alcohols), ethyl ether, acetic acid, chloroform, as well as surface tension depressants (wetting agents as "tergitol" and soap) were used. These were applied in various sublethal concentrations in water prior to the use of a strongly hypertonic solution as well as mixed with hypertonic solutions. These experiments gave negative results, which may indicate that the protoplasts were permeable rather than impermeable. Again, some of the permanently refractory species such as *Leucodon, Grimmia, Ulota,* and *Hedwigia* take up water when dry and become apparently "turgid" with almost startling rapidity. The plasma membrane does not retard the rapid entrance of water into these plants. It is thus assumed that the protoplasts are not impermeable to water.

On the other hand, failure to plasmolyze in hypertonic solutions may indicate that the plasma membrane of refractory species is highly permeable not only to water, but to solutes as well. Several investigators have reported that mature sieve tubes are highly permeable and fail to be plasmolyzed by the usual hypertonic solutions, *e.g.,* Crafts (1939). Esau (1939) summarizes the literature on phloem permeability. Failure to plasmolyze in hypertonic solutions was the principal evidence obtained for permeability in these instances. Crafts (1939) reports permeability in *Polytrichum,* presumably in the leptome tissue of the gametophytic stem.

Holle (1915) has shown that air-dry plants of *Mnium affine,* and to a less marked extent, *Catharinea* (*Atrichum*) *undulatum,* when placed in hypertonic solutions, failed to plasmolyze, but became hydrated as if they had been placed in water. The same species when freshened from the dry state by immersion in water prior to treatment with hypertonic solutions plasmolyzed normally. He attributes this behavior to the permeability of the dry protoplast. Höfler (1918) confirmed Holle's observation by showing that *Bryum capillare* behaved in the same manner as Holle's *Mnium affine.* The writer has confirmed Höfler's observation for *B. capillare* and has found that *Mnium cuspidatum* behaved in the same way. This type of behavior is unusual and its extent among bryophytes is not known.

For *B. capillare* and *M. cuspidatum,* the lengths of the intervals prior to complete hydration are directly proportional to the solution concentrations. When these species are moistened prior to immersion in solutions, concentrations of KCl at or above 0.3 N, and of sucrose at or above 0.5 molar are hypertonic for *M. cuspidatum;* concentrations of KCl at or above 0.4 N are hypertonic for *B. capillare.*

Holle showed for *M. affine* that, after preliminary hydration without plasmolysis in a given hypertonic solution, plasmolysis would occur if it were placed in a second solution of the same substance at a much higher concentration. Our experiment below confirms and extends Holle's observation. The current season's vegetative shoots of *B. capillare* and stolons of *M. cuspidatum* were used. The procedure followed was the same for both species. Shoots were removed from the air-dry plants and a dozen or more of these were placed in each of a series of KCl solutions. The protoplasts became hydrated without the occurrence of plasmolysis. The cells appeared to be fully expanded after 2–5 hours in hydrating solutions of 0.6 N and lower. The time required for complete hydration in 1.0 N KCl and higher may be 10–12 hours or longer. The cells gradually became moribund in hypertonic solutions, and the more concentrated the solution, the more rapidly this occurred. Thus the results with the use of high concentrations were not reliable.

Shoots hydrated in one of the lower concentrations were divided between a second series of concentrations of the same salt. These second solutions are called the plasmolyzing solutions. After 20–30 minutes, examinations were made to determine at which concentration plasmolysis occurred. This was repeated for the samples in each of the other hydrating solutions. Table 3 summarizes the results.

It will be noted that when dry *B. capillare* was hydrated with a normally hypotonic solution of KCl, plasmolysis occurred in the plasmolyzing solution between 0.4 N and 0.5 N. When hypertonic hydrating solutions were used, plasmolysis occurred in the plasmolyzing solutions at 0.2 N greater concentration than the corresponding solution used for hydration. *M. cuspidatum* behaved similarly, with the difference that the concentration of the plasmolyzing solution was 0.3 N greater than that of the corresponding hydrating solution.

TABLE 3. *Plasmolysis in certain dry mosses. The mosses were first hydrated with KCl solutions without plasmolysis. When these were placed in higher concentrations of the same salt, plasmolysis occurred as indicated* (+).

First or hydrating solutions →	0.2N	0.3N	0.4N	0.5N	0.6N
		Second or plasmolyzing solutions			
Bryum capillare,					
normal osmotic value, 0.35N.....	0.2N—				
	0.3N—	0.3N—			
	0.4N—	0.4N—	0.4N—		
	0.5N+	0.5N+	0.5N—		
		0.6N+	0.6N+		
			0.7N+		0.7N—
					0.8N+
					1.0N+
Mnium cuspidatum,					
normal osmotic value, 0.25N.....	0.2N—				
	0.3N—	0.3N—			
	0.4N—	0.4N—	0.4N—		
	0.5N+	0.5N—	0.5N—	0.5N—	
	0.6N+	0.6N+	0.6N—	0.6N—	
		0.7N+	0.7N+	0.7N—	
			0.8N+	0.8N+	
				0.9N+	

This experiment shows that the hydrating protoplasts of these species are highly permeable to KCl. Entrance of salt is sufficiently rapid to prevent plasmolysis. It shows further that, sometime after hydration, the plasma membrane becomes markedly impermeable to KCl.

A similar experiment with *M. cuspidatum* using sucrose in a series of 0.1 molar concentration intervals showed no plasmolysis in the hydrating solutions and little or no plasmolysis in the plasmolyzing solutions. Thus with this procedure the protoplasts remained highly permeable to sucrose.

The bearing of these experiments on the question of the permeability of the refractory species can be only inferential. Immersion of *Mnium* and *Bryum* when dry in hypertonic solutions demonstrated an initial temporary permeable period. The dry, permeable protoplasts of these mosses attained semipermeability rapidly when they were placed in water. The bryophytes of type 2 may be characterized by delay in the complete recovery of semipermeability in water after a dry period. Type 2 would thus extend the behavior of dry *Mnium* and *Bryum* in hypertonic solutions to a similar though partially permeable behavior when in water. Type 3 bryophytes would then represent the behavior of type 2 in greater degree. Type 3 species would have a temporary permeability in water similar in degree to dry *Mnium* and *Bryum* in hypertonic solutions. Type 4 might be considered to have attained the logical extreme in this tendency where the protoplast hydrated in water retains the permeability of the desiccated protoplast continuously. However, this hypothesis does not explain satisfactorily the aberrant type 5, particularly the hydrophyte *Fontinalis*, which may become refractory without previous drying. Thus other factors besides desiccation may induce this presumed permeability.

The refractory nature of different bryophytes toward plasmolysis in hypertonic solutions seems to be an expression, in different degrees, of a single type of behavior. Refractoriness is at first conditioned by a previous drought period and culminates in types in which this property is permanently established. Several factors that might possibly contribute to this refractoriness have been discussed briefly. Some of these have been dismissed because of their apparent inapplicability. The greatest amount of evidence, although indirect and thus inconclusive, supports the view that this series of behavior patterns toward plasmolyzing agents is due primarily to temporary or permanent permeability of the protoplasts to solutes. Further, the physical state of the cytoplasm may, in some instances, alter typical plasmolytic response and thus be a second contributory cause to refractoriness.

SUMMARY

The osmotic values of a number of bryophytes were determined with the view of possible correlation with habitats. Correlation with the light intensity of these generalized habitats and not with the available moisture was indicated.

Several types of behavior toward plasmolyzing agents were found among the forms studied. The first type, considered typical, included species which became plasmolyzed, when wetted after a previous dry period, at the same value as when tested after a prolonged moist period. A second type showed some temporary refractoriness toward plasmolyzing agents after a dry period. These plasmolyzed at a somewhat greater concentration than when tested in a fresh state after a moist period. A third type showed a marked temporary increase of this refractory property. A fourth type failed to plasmolyze typically at any time after a dry period. A fifth type showed an aberrant behavior, where identical samples at different times became plasmolyzed or failed to become plasmolyzed.

A number of possible contributory causes to this refractory behavior were examined briefly. Indirect evidence points to protoplasmic viscosity and permeability as being principally involved.

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THE CYTOLOGY OF POLYPLOID SORGHUM [1]

T. C. Chin[2]

GRAIN SORGHUM (*S. vulgare*, var. *hegari*) was treated with colchicine in the spring of 1945. Three methods were used: (1) cotton soaked with an 0.4 per cent aqueous solution of colchicine was packed around the crown of young seedlings and kept moist by adding more solution, (2) germinating seeds were alternately placed in an 0.1 per cent colchicine solution for 24 hours and then in water for 24 hours, (3) an 0.4 per cent colchicine lanolin emulsion containing a small amount of aerosol was smeared around the crown of the seedling. For the first method two and one-half days were sufficient, for the second method six days were most effective, while for the third method daily applications for three days were adequate. The third method proved to be the cheapest, most convenient and most effective treatment.

These treatments produced both tetraploid and octoploid plants. The polyploids were stouter and shorter than the diploids. The time of flowering was delayed in the polyploids. The tetraploid plants flowered about twenty days later, and the octoploids more than a month later, than the diploids.

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TABLE 1. *Total configurations in the tetraploid sorghums.*

Combinations	Frequency
6(IV) + 8(II)	4
4(IV) + 1(III) + 10(II) + 1(I)	1
4(IV) + 12(II)	3
3(IV) + 1(III) + 12(II) + 1(I)	1
2(IV) + 16(II)	4
1(IV) + 18(II)	2
20(II)	2
19(II) + 2(I)	1
Number of cells observed	18

The cytological analysis of meiotic metaphase was made to compare chromosome behavior in diploids and autopolyploids. The anthers were fixed in acetic-alcohol and stained with acetocarmine.

The chromosomes of the diploid plants usually paired as bivalents although rings and chains of four chromosomes were found occasionally. Most of the bivalents were associated as rings with two terminal chiasmata at metaphase. In 18 per cent of the bivalents there were three chiasmata, while less than one per cent were rod bivalents associated by a single chiasma.

TABLE 2. *Association of chromosomes in octoploid sorghum.*

Valance No. of chiasmata		VIII	VI	V	IV	III	II	I	Total no. configurations	Total. no. chiasmata
9		2							2	18
8		14							14	112
7			3						3	21
6			27		2				29	174
5			6	3	8				17	85
4				2	98				100	400
3					11	2	112		125	375
2						6	278		284	568
1							12		12	12
0								7	7	0
Total number		16	36	5	119	8	402	7	593	1765
No. per cell	Ave.	0.8	1.7	0.2	5.7	0.4	19.1	0.3	...	84.0
	Range	0–4	0–5	0–2	0–12	0–2	8–36	0–2		
Percentage of chromosomes		7.6	12.9	1.5	28.3	1.4	47.9	0.4		
Total number of cells observed										21

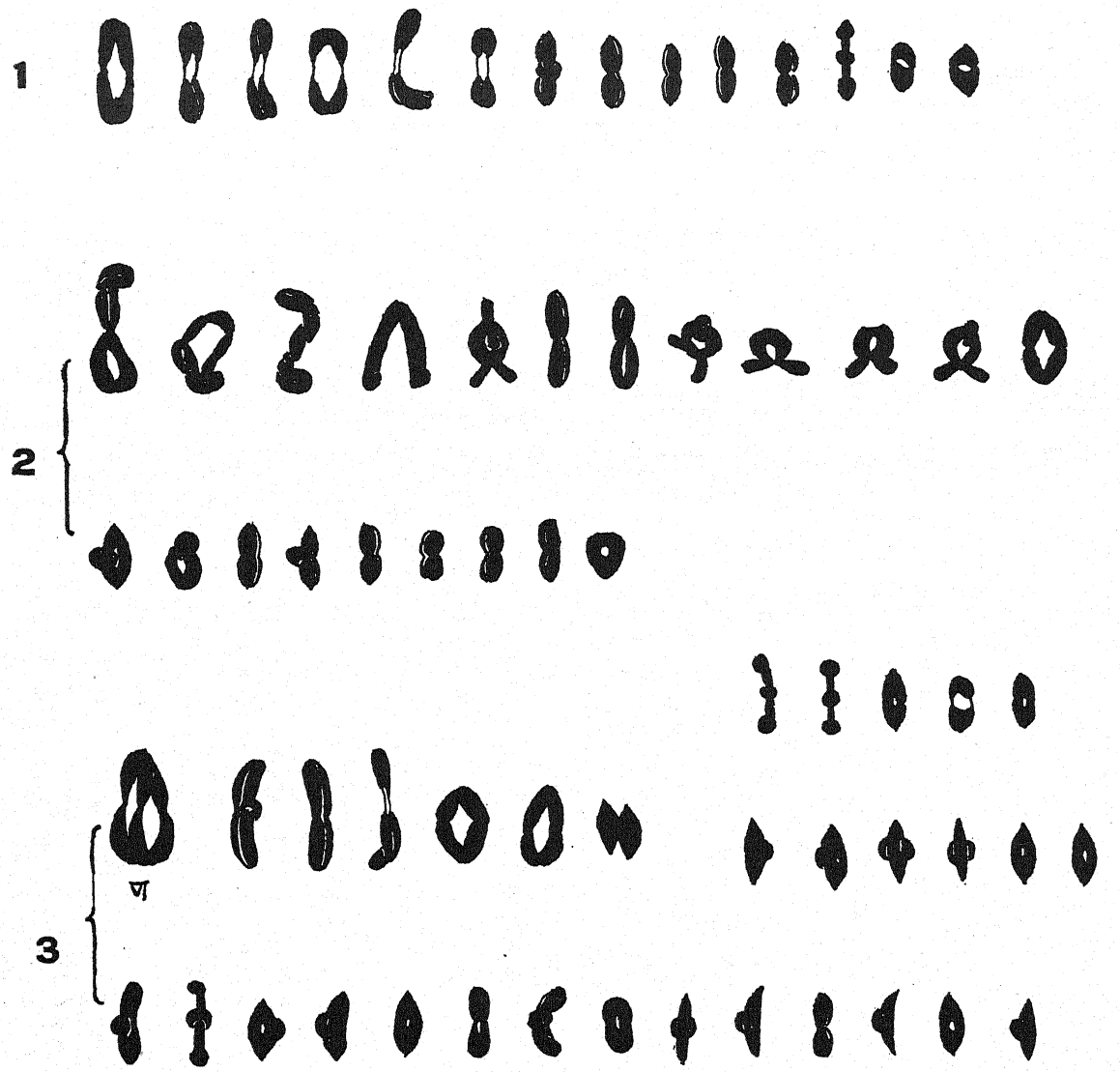


Fig. 1–3.—Fig. 1. Meiotic metaphase chromosomes of the tetraploid sorghum, showing 6(IV) + 8(II). ×900.—Fig. 2. Meiotic metaphase chromosomes of the octoploid sorghum, showing 2(VIII) + 3(VI) + 7(IV) + 9(II). ×900.—Fig. 3. Meiotic metaphase chromosomes of the octoploid sorghum, showing 1(VI) + 6(IV) + 25(II). ×900.

TABLE 3. *Frequency of chiasmata per cell.*

8n				4n		2n	
No. of chiasmata	No. of cells	No. of chiasmata	No. of cells	No. of chiasmata	No. of cells	No. of chiasmata	No. of cells
79	1			37	2		
80	4			38	2		
81	4			39	3		
82	1			40	3		
83	2			41	3		
84	2			42	1	20	9
85	0			43	1	21	3
86	2	90	1	44	1	22	1
87	1	91	1	45	0	23	2
88	0	92	1	46	1	24	3
89	0	93	1	47	1	25	2
Number of cells observed			21		18		20
Mean number of chiasmata per cell and S. E.		84.0 ± 0.95		40.7 ± 0.67		21.7 ± 0.43	

Most of the meiotic chromosomes in the tetraploid plants were paired as bivalents, but there were an average of three quadrivalents per cell. Trivalents and univalents were rare. The various combinations of meiotic configurations found in 18 cells are shown in table 1 and figure 1.

In the octoploid plants the meiotic chromosomes were found most frequently as bivalents. The average frequencies per cell of the various types of association were 0.8 octovalents, 1.7 sexivalents, 0.2 quintavalents, 5.7 quadrivalents, 0.4 trivalents, 19.1 bivalents and 0.3 univalents. The relative frequencies of octovalents and sexivalents were much higher than were found in octoploid *Petunia* by Levan (1939). The chiasma frequencies and chromosome associations for 21 cells are shown in table 2 and in figures 2 and 3.

Since most of the chiasmata of the meiotic chromosomes of sorghum are terminal or subterminal the bivalents and multivalent associations are relatively simple. In comparing the quadrivalents of the octoploid with those of the tetraploid it was found that there were more variations in the octoploid (fig. 1, 2, 3).

Polyploidy is often associated with a decrease in chiasma frequency due presumably to greater competition in chromosome pairing (Upcott, 1939). In sorghum, however, there is no significant change in chiasma frequency as the chromosome number is increased. The frequency of chiasmata per cell for diploid, tetraploid and octoploid plants are shown in table 3. The correlation ($r = -0.22 \pm 0.22$) between chiasma frequency and the number of quadrivalents per cell indicates that quadrivalent formation has little or no effect on chiasma frequency. In autotetraploid barley, however, there is a significant negative correlation ($r = -0.55 \pm 0.16$) between quadrivalent formation and chiasma frequency (Chin, unpublished data).

A comparative survey of chiasma frequency in diploid and tetraploid plants does show a general decrease in chiasma frequencies per chromosome in the polyploid forms. An analysis of *Solanum Lycopersicum, Primula kewensis, Campanula persicifolia, Allium schoenoprasum,* and *Primula sinensis* (Upcott, 1939); *Secale cereale* (Chin, 1943), *Sorghum vulgare* and *Hordeum vulgare* (Chin, unpublished), *Tradescantia virginiana* (Anderson and Sax, 1936) and *Brassica oleraceae* (Howard, 1939) show reduction factors for chiasma frequencies in the tetraploids of 0.94, 0.94, 0.83, 0.77, 0.89, 0.91, 0.94, 0.82, 0.76 and 0.96 respectively. There is no consistent relation between the reduction factor and either chromosome size or chromosome number although the genera with the largest chromosomes do have low reduction factors.

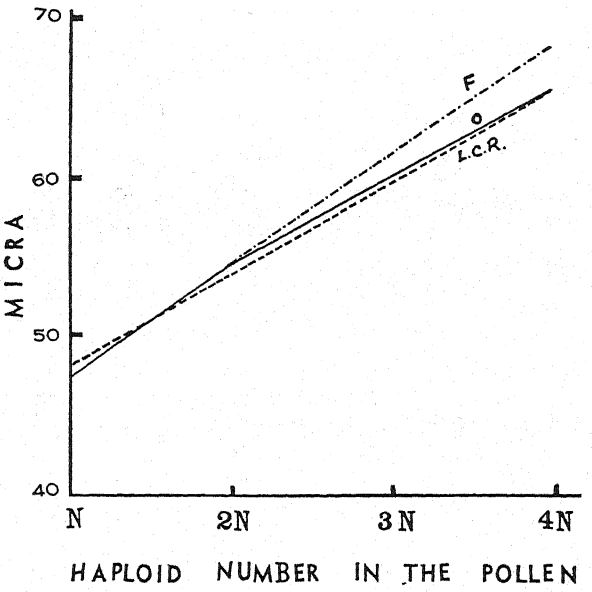


Fig. 4. Relationship between the number of haploid sets of chromosomes and the diameter of pollen. O, observed; F, calculated according to the formula $\sqrt{D_1}$ and L. C. R., calculated linear coefficient of regression.

Polyploidy usually results in larger cell size (Belling and Blakeslee, 1923; Blakeslee and Avery, 1937; Randolph *et al.*, 1944; Darlington, 1937). There is a high degree of correlation between chromosome number and size of pollen grain in the genus *Sorghum*. The data and calculations are shown in table 4.

The pollen diameters in the polyploid series fit a linear coefficient of regression. For each increase of haploid set of chromosomes in the pollen grain the increase in diameter was found to be equal to the square root of the diameter of the haploid pollen grain. Thus, $D_n = D_1 + (n - 1)\sqrt{D_1}$, assuming D_n to be the diameter of pollen with n haploid sets

TABLE 4. *Size of pollen of the diploid, tetraploid and octoploid sorghum.*

Haploid no. in pollen		n	2n	3n	4n
Diameter	Observed	47.4	54.4		65.3
	Expected [a]		54.3	61.2	68.0
	(Micra) [b]	47.8	53.6		65.4
Volume	Observed	55764.20	84297.96		145800.45
	Expected [a]		84962.34	114160.48	143358.62
	(Cubic micra) [b]	54635.93	84649.24		144972.84

[a] Calculated according to formula mentioned in this paper.
[b] Linear coefficient of regression.

of chromosomes and D_1 to be the diameter of the haploid pollen grain.

The volume of the pollen can also be used as a prediction of chromosome number in the pollen grain. With every addition of a haploid set of chromosomes there is an increase of $\pi/6 \times V_1$, assuming V_1 as the volume of the haploid pollen grain. The volume of pollen with n sets of chromosomes can be calculated as $V_n = (n - 1)\ \pi/6 \times V_1$ and $V_2 = (1 + \pi/6)\ V_1$ for tetraploids and $V_4 = (1 + \pi/2)\ V_1$ for octoploids. The relations between chromosome number and pollen grain diameter are shown in figure 4, and the relation between chromosome number and pollen grain volume is shown in figure 5. This relationship varies in different genera and no general formula is applicable to all organisms.

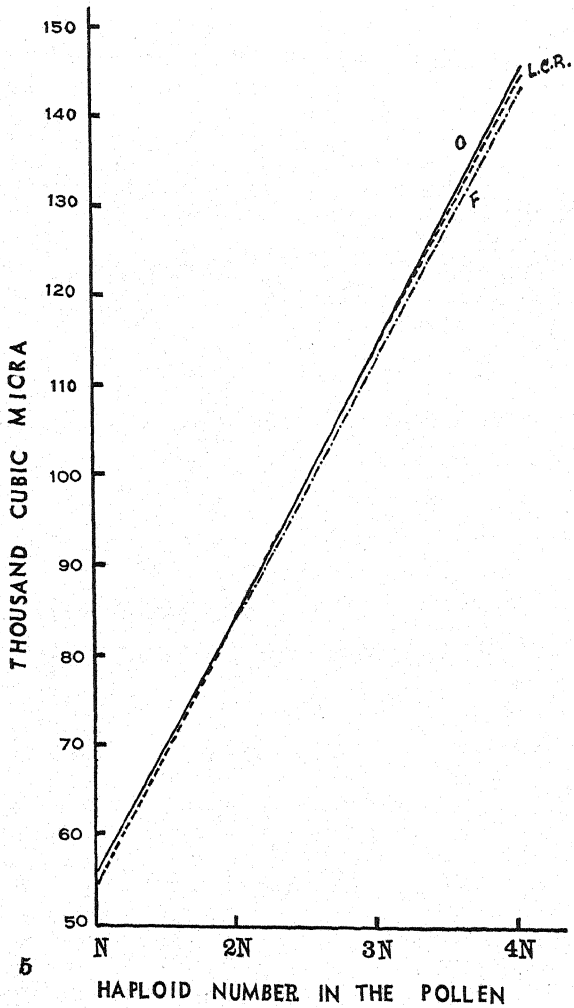


Fig. 5. Relationship between the number of haploid sets of chromosomes and the volume of pollen. O, observed; F, calculated according to the formula of $\pi/6 \times V_1$ and L. C. R., calculated linear coefficient of regression.

The percentage of sterile pollen, as measured by obviously imperfect pollen grains, is 19 per cent in the 4n and nearly 80 per cent in the 8n sorghums. The increased sterility of autopolyploids is due largely to irregular segregation of some of the chromosomes at meiosis. Variation in irregular meiotic behavior in autopolyploids has been related to chromosome length by Kostoff (1940) although Müntzing (1936) and Randolph (1941) find little or no relation between chromosome length and pollen sterility.

The partial sterility of newly formed autopolyploids makes their commercial utilization of doubtful value where seed production is essential. Not only are our polyploid sorghums partially sterile, but they are late in maturity. Unless they can be improved by hybridization and selection they are not likely to be of economic value. They may, however, be of some value in hybridizing with related genera where chromosome balance may play some par in compatability.

SUMMARY

Tetraploid and octoploid forms of sorghum (*S. vulgare* var. *hegari*) have been induced by colchicine treatment. The numbers of chiasmata per chromosome are essentially the same in the 2n, 4n, and 8n forms. There is no correlation between quadrivalent formation and chiasma frequency in the tetraploid. Pollen grain size is proportional to chromosome number. The relative sterility of polyploid sorghums makes them of doubtful economic value.

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A DEVELOPMENTAL STUDY OF MAIZE WITH PARTICULAR REFERENCE TO HYBRID VIGOR [1]

Harry Lloyd Weaver

THE PERPLEXING phenomenon of hybrid vigor has been investigated in detail in comparatively few groups of plants and animals, although its widespread occurrence is a matter of common knowledge. Most studies have been concerned with the genetical and physiological aspects of the problem, while the internal anatomy of inbred and hybrid plants during the course of their development from germination of the seeds to maturity of the plants has received relatively little attention. The present anatomical investigation, using two inbred lines of maize and their first-generation hybrid, was made with the hope that such a study might contribute to a better understanding of the general problem of hybrid vigor.

During the past 25 to 30 years many workers have investigated hybrids showing augmented vigor and two important hypotheses to explain the reason for hybrid vigor have been formulated. One attributed hybrid vigor to a stimulatory physiological effect of heterozygosity ("heterozygosis") (Shull, 1911), while the other attempted to explain the phenomenon as dependent upon the action of linked dominant or partially dominant factors from the two parents brought together in the hybrid (Jones, 1917). More recently, Jones (1945) has interpreted hybrid vigor as "an accumulative effect of favorable heredity from both parents . . . . even when only single allelic differences are involved provided genes have multiple effects."

The word "heterosis" was proposed by Shull (1914) as a simple means of describing hybrid vigor as the result of some extra-genetic stimulus produced by bringing together unlike genetic complements. The term has since been adopted generally, and is now in quite common use as a designation of hybrid vigor, regardless of the cause.

While numerous investigators have rejected the "linkage-dominance" hypothesis, it has been more generally accepted by students of hybrid vigor than has the heterozygosis explanation. The problem is not solved, however, and during the past few years the scope of investigation has widened. One important aspect of the study has to do with the role played by embryo size.

Embryo size as an indication of heterosis.—Based upon the results obtained with maize and tomatoes, Ashby (1937) and Luckwill (1939) concluded that the relative growth rate of the hybrid did not differ from that of its more vigorous parent but that greater initial embryo volume, growing at a rate which appeared to be inherited as a Mendelian dominant from the more vigorous parent, resulted in mature hybrid plants which weighed more than twice as much as the larger parent. Findings of other workers, notably East (1936) and Shafer (1944), are not in accord with this conclusion, and Hatcher (1940) showed that embryo size was not always the determining factor in final size of hybrid plants.

Anatomical investigations.—As mentioned previously, comparatively little attention has been given to the internal anatomy of hybrid plants and their parents. The studies that have been made, however, have contributed important information concerning heterosis.

Studies of cell-size relationships between hybrids and inbreds have rather definitely established the fact that increased size of hybrids is not due to greater cell size. Kostoff and Arutiunova (1936) state that hybrids of *Nicotiana* and *Solanum* with increased vigor failed to show larger cells than the parent lines, while hybrid embryos of tomato studied by Ashby (1937) were larger but were made up of cells of the same size as those of the inbred embryos. Except at the highest level of nitrogen used, medulla cells of hybrid maize stems studied by Burkholder and McVeigh (1940) were intermediate in size between those of the two parents. Hybrid plants grown in solutions containing 4.0 milligram atoms of nitrogen per liter had medulla parenchyma cells in the third internode larger than those of either parent at the same level on the plant.

Plumular meristem size in heterotic maize was found to be no greater than that of the larger parent while, in one pedigree, nuclear size in the plumular meristems of the hybrid was significantly greater than in the larger parent (Bindloss, 1938). Whaley (1939b) found no relationship between volume of the meristem and degree of hybrid vigor in the embryo or during development of tomatoes. He further observed that cell and nuclear size decreased during development, but that this decrease took place more rapidly in the inbred parents. In an earlier paper, Whaley (1939a) reported that heterosis produced its effect on total plant size of tomatoes. Size of fruits was not increased by heterosis, but they were produced in greater number.

The most complete histological investigation of hybrid vigor that has come to the attention of the writer is that of Kiesselbach (1922) with maize. Seven pure lines and ten hybrids were studied at maturity and the hybrids were compared with the average of the two parent lines. The points recorded, with percentage increase or decrease shown

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The writer wishes to express to Prof. Edmund W. Sinnott his sincere appreciation for the generous assistance and counsel received during the course of the investigation and preparation of this paper. Thanks are tendered also to Dr. D. F. Jones, of the Connecticut Agricultural Experiment Station, for making available the facilities of the Station's farm, and to Prof. R. A. Emerson, of Cornell University, for the seed used in the investigation.

by the hybrids, were as follows: stalk diameter, 46 per cent greater; number of fibrovascular bundles in stem cross section, 45 per cent greater; number of bundles in one square centimeter of stem cross section, 34 per cent less; number of bundles occurring along one diameter of stem cross section, 21 per cent greater; number of bundles along one centimeter of stalk diameter, 19 per cent less; actual diameter of vascular bundles, 15 per cent greater; number of medulla cells along one diameter of stem cross section, 38 per cent greater; average diameter of medulla cells, 10 per cent greater; leaf thickness, 14 per cent greater; average thickness of upper and lower epidermal cells, 4 per cent greater; number of vascular bundles per centimeter cross section of leaf, 8 per cent less; average width of epidermal cells on upper and lower leaf surface, 4 per cent greater; and average leaf area, 46 per cent greater. Material for stem studies was taken midway of the first internode above the ground, and that for leaf study was taken at the widest portion of the main-ear leaf midway between the midrib and the margin.

Paddick (1944) studied two inbred lines of maize and their reciprocal hybrids and found that the hybrids developed faster, made greater overall growth, and were composed of larger plant parts, particularly leaves. He noted further that the inhibitory effect of tassel formation and rapid culm development on continued leaf development was less effective on the hybrid plants or did not take effect so soon as on the inbred parents. He concluded that "hybrid vigor appears as a factor both accelerating the growth activities of the plant and carrying them on to a point beyond that common in less vigorous inbreds."

Comparisons of inbreds and hybrids at maturity, while of great value, nevertheless leave a large gap in our knowledge as to how differences in size and internal anatomy arise during development. Accordingly, the present investigation was not confined to a study of one level only of mature plants. Instead, entire plants were studied throughout their development.

Materials and methods.—Two inbred strains of maize and their first-generation hybrid were grown in the field at the Mount Carmel station of the Connecticut Agricultural Experiment Station during the summer of 1940. Two plantings, the first on June 2, 1940, and the second on July 3, 1940, were made. The seed was furnished by Prof. R. A. Emerson of Cornell University. The lines will be designated in this paper as Co. 1, Co. 2, and Co. 2 × Co. 1. Only one plant was permitted to develop in each hill and the hills were twelve inches apart in rows three feet apart, thus allowing the plants to grow to their maximum normal size.

Samples were taken at four-day intervals from the third day to the fifty-eighth day after planting of the seeds. Three plants, from a total population of 90 to 100 plants of each strain, were selected at each sampling period, care being taken to pick plants average in size and general vigor, and the leaves were measured and recorded in sequence from the base of the plant toward the tip. Measurements consisted of length of the leaves from the ligule to the tip and the width at the widest point, all measurements being made before any wilting took place. At the tips of immature plants, no measurements were made of leaves which, because of their small size, could not be removed entire. Likewise, at the base, dried leaves could not be recorded, but all leaves that could be removed were preserved for dry weights, those from each plant being weighed together after drying for twenty-four hours at 100°C.

After removal of the leaves, stem height was measured and, where possible, all internodes were measured for length, to the nearest half millimeter. From the point of attachment of a leaf base to the point of attachment of the next leaf base above was arbitrarily considered a complete internode. By this method of measurement each internode included approximately half of the nodal area at its base and approximately half of the nodal area directly above, thus eliminating the necessity for distinguishing between nodal and internodal tissues but still furnishing measurements which were constant enough to permit comparisons.

During the early stages of growth, the entire stem of each plant was preserved after removal of the leaves. As the internodes elongated it became necessary to preserve only a portion taken from the center of each expanded internode. All stem material was killed and fixed in CRAF solution (Randolph, 1935) for 24 hours, after which it was washed thoroughly with 70 per cent ethyl alcohol and stored in the same grade of alcohol.

Because of the great amount of time consumed in preparing slides and making measurements from them, it was not possible to investigate stem structure at each four-day interval. Accordingly, four ages (3, 14, 38, and 58 days after planting) were selected for this study.

Transverse serial sections of the entire stem of one plant of each strain at 3 days and at 14 days after planting were cut by the paraffin method. At the 38- and 58-day stages, only the young tissue at the tip was cut in paraffin. For older tissues, sections were made with the freezing microtome from samples taken at the center of each internode. Complete cross sections of the internode were cut in every case and 10 per cent gum arabic was used as the matrix for sectioning with the freezing microtome. Sections made by the paraffin method as well as those made with the freezing microtome were stained with a combination of safranin and fast green, mounted upon microscope slides, and sealed with clarite.

From these slides a series of drawings was made. A complete cross section of each internode of each of the plants at four ages (3, 14, 38, and 58 days after planting) was outlined on paper, and within each outline every vascular bundle or provascular strand cross section was traced. The bundle sheath

and all tissues within it were included within the bundle outline. Various magnifications were necessary, depending upon the size and stage of development of the internode. For all internodes,[2] the stem cross sections were projected upon heavy paper at various magnifications by means of a Spencer delineascope for magnifications of 39 diameters for mature internodes, and with a Bausch and Lomb vertical-projection microscope for magnifications of 100, 150, 350, and 1720 diameters for immature internodes. In the delineascope, the slides were held for projection in an adaptor fashioned from two lantern-slide cover glasses held apart by strips of blotting paper at the ends and bottom edge and bound in the same manner as a lantern slide, but with the top edge left open to permit insertion and removal of the slides.

From these drawings, number of vascular bundles, internode cross-sectional area, and total cross-sectional area of vascular bundles were computed for each internode of each plant at the four different ages. To obtain area measurements of the internode cross sections, the drawings were cut out and weighed on an analytical balance. The outline tracings of all vascular bundles and provascular strands were then cut out and weighed as a unit for each internode cross section. Weights were then converted to areas in square millimeters as follows: The weight of the cutout was divided by the weight of the standard and the quotient was multiplied by the area of the standard (10,000 square millimeters) to give the area, in square millimeters, of the cutout. This value was then divided by the square of the diameter magnification to give the actual area, in square millimeters, of the object. The standard was a piece of heavy paper measuring one hundred millimeters on a side (10,000 square millimeters) and was selected as follows: Twenty-six squares of paper one hundred millimeters on a side were cut from the margins of the paper outside of twenty-six of the internode drawings. The drawings from which the samples were taken were picked at random from the total of 114 sheets comprising the entire set. These squares were weighed individually to tenths of milligrams, the weights of the twenty-six samples, ranging from 801.0 to 860.6 mg., were averaged and the one (830.1 mg.) which most closely approximated the average weight was selected as the standard. In order to eliminate errors resulting from changes in atmospheric moisture, the standard was kept at all times under the same conditions as the cutouts to be weighed and was weighed each time a set of cutouts was weighed. The weight of the standard at the time of weighing any particular set of cutouts was used in calculating the areas for that set. While areas computed in this manner are not very accurate as absolute values, for comparative purposes they appear to be valid.

For studies of cell size of medulla, sieve tubes, and metaxylem, outline drawings were made with the aid of the projection microscope and areas in square microns were determined by use of a planimeter. Twenty pith cells from the center of the internode cross section were drawn and measured at three levels (base, midway of the stem, and tip) on each plant at each of the four ages. Ten sieve-tube cells and ten metaxylem cells from each internode where they were differentiated in each of the plants at 14, 38, and 58 days after planting were drawn and measured.

RESULTS.—*General developmental pattern of the corn plant.*—Since an understanding of normal development must necessarily precede any investigation of extraordinary growth phenomena, the findings of various workers, together with certain other data which appeared in the present investigation seem important as background. The maize stem follows the general pattern of the "palm type" of von Mohl as modified by DeBary (1884), and investigated by Strasburger (1891), with all fibrovascular bundles representing leaf or secondary axis traces which curve into the stem from the point of entry at the base of the leaf or branch and pass downward through several internodes before they coalesce with bundles entering from lower leaves. Sharman (1942) traced median vascular bundles through eight or more internodes in young shoot parts of maize, while Esau (1943) reports the same observation and reiterates the classification of the maize stem as the palm type.

Transverse sections of the meristematic stem tip used in the present study indicated that the bundles arise as patches of small, densely cytoplasmic cells and gradually increase in size and complexity in the definite manner described by Hershey (1935), resulting at maturity in the typical closed maize bundle.

This study also confirms the findings of Martin and Hershey (1935) that maximum development of a maize plant is determined largely during a formative period comprising the first 30 to 40 days after germination. During the first 38 days after planting, almost all structures of the plants were formed. All nodes, internodes, leaves, axillary buds, tassel, at least five whorls of permanent roots, and most of the vascular bundles could be identified at the end of that period, but some differentiation continued and was still going on in the upper internodes at 58 days, the date of final sampling. Martin and Hershey (1935) state that an additional period of 35 to 40 days elapses before differentiation is complete.

Several features of the general developmental pattern in maize, previously mentioned by other

[2] Throughout the following report, the basal internode of the stem proper has been designated as internode number two, the so-called "mesocotyl" being considered the first internode of the plant, and successively higher internodes were numbered consecutively. Internodes below the seventh from the 58-day and 38-day plants and below the fifth from the 14-day plants were not studied, due to the confused pattern of vascular elements in these shorter internodes. Because of the specialized anatomy of the first internode (Avery, 1930), it has not been included in the study of any of the plants.

workers, were again observed during this investigation.

Ratio of leaf length to leaf width showed a steady increase during the development of the maize leaf. Abbe, Randolph, and Einset (1941), in a study of leaf-blade pattern in relation to shoot apex, made a similar observation. At the earliest stage at which leaves could be removed from the plant, length was approximately twice the width, but growth in length took place at a much more rapid rate than growth in width, resulting at maturity in the familiar linear leaf of maize with length ten to twelve times the width. During development of the plant, progressively older leaves from the apex toward the base showed this constant increase in length as compared with the increase in width. At 38 days after planting, leaves four to nine inclusive had reached maturity and the ratio of length to width was the same for all. Less mature leaves above the ninth were progressively shorter as compared with their width. At 58 days, all leaves had reached approximate maturity and exhibited a constant relationship between length and width. It appears, therefore, that leaf shape during development and at maturity in the maize lines studied is definite. Dry weight of leaves, when all leaves of a plant were weighed together, showed the expected constant, exponential increase as the age of the plants increased.

A definite pattern seemed also to govern the length of individual internodes which make up a maize plant. With internodes, however, length at maturity was related to position within the stem. Beginning with the short internodes at the base of the stem, there was a progressive increase in length up to an approximate half-way point. Above that point, length of individual internodes decreased with each successively higher internode. L. B. Abbe (1936) presents graphic illustrations of this pattern in several different maize lines.

During development, the basal internodes reached full length earliest, with full extension progressing upward with increasing age of the plant. Growth in height of the plant as a whole took place at a steady, exponential rate. Cross-sectional area of successive internodes decreased from base to apex of the plant in all internodes of all plants studied at the four ages (3, 14, 38, and 58 days after planting). It should be noted, however, that cross-sectional area of the internodes was not related to their length.

During the formative period from germination to 38 days after planting, average cross-sectional area of vascular bundles showed the progressive increase which was anticipated in successively older internodes from the apex of the plant toward the base. At 58 days, however, average cross-sectional area of bundles decreased more gradually from the apex downward, and from about the thirteenth internode and below, the average cross-sectional area of bundles was nearly constant. This definite size in any one of the three lines differed from the other two but there was no significant variation within a particular line, indicating that a given race of maize has a characteristic bundle size at maturity, at least in the lower two-thirds of the stem. In the material studied at 58 days after planting, the internodes above the thirteenth were still somewhat immature, so it is possible that the vascular bundles in those internodes might have reached the same dimensions as those in the lower internodes. It seems more probable, however, that at maturity they would have been somewhat smaller, since the upper internodes presumably grow at the same rate as the rest of the plant but are formed later than the lower internodes and, therefore, have a shorter period of growth.

Bundle number also exhibited a progressive increase from the apex toward the base during development of the plants after germination to 38 days after planting, but bundle number increased only slightly between 38 and 58 days after planting, again illustrating the fact that nearly all structures of the plant are present at the end of 30 to 40 days after planting. At 38 days and 58 days after planting, the increase in number of vascular bundles from the apex toward the base of the plant extended only to the thirteenth internode. Below that the number was constant due, possibly, to the fact that the bundles extended downward through a more or less definite number of internodes before anastomosing with other bundles, affecting bundle number in the following manner: the number of bundles supplied to an internode by the leaf immediately above is increased by bundles from leaves higher up, but in any one internode only a certain number of leaves have contributed, the bundles from leaves too far above having coalesced with other bundles before they reached the internode under consideration. By this arrangement, it is to be expected that only the internodes from the base upward to a certain point on the plant would show equal numbers of bundles. Above that point the number would decrease progressively in successive internodes as the number of leaves between the given internode and the tip of the plant decreased. The constant number of bundles from the base to the thirteenth internode and the progressive decrease above that point in the plants studied at 38 and 58 days are an illustration of such a pattern.

Degree of hybrid vigor in the material studied.— A high degree of hybrid vigor was apparent in hybrid Co. 2 × Co. 1 throughout development of the plants from germination of the seeds to maturity. The hybrid seeds germinated two days earlier than those of the two parents and the plants were taller and had larger leaves at all stages of development. The extent to which greater bulk of hybrid plants occurred, as compared with inbred plants, is revealed by measurements made of stem height, cross-sectional area of internodes, and dry weight of leaves at intervals during the life of the plants.

Comparative stem heights, measured from base to tip with all leaves removed, showed the hybrid to be taller than either parent at all ages from 22 days after planting, the earliest age at which stem height could be measured, to 58 days, when tassels were well developed and silks were just appearing. All internodes (except No. 9 at 22 days and No. 10 at 46 days) contributed to the greater height of the hybrid. At 58 days, internodes 13 to 19 of parent Co. 2 were longer than the corresponding ones of the hybrid, but the latter plant attained greater total height from greater length of internodes below the thirteenth. Similarly, the upper internodes of parent Co. 2 at 58 days were greater in cross-sectional area than those of the hybrid, while at all other ages the hybrid was larger in cross-sectional area of all internodes, with the exception of internode five at three days after planting.

Total mass of leaves also reflected the greater vigor of the hybrid. Dry weight of leaves of the hybrid plants was significantly greater at all ages during development, and the percentage advantage shown by the hybrid at 14 days was maintained at an essentially constant level throughout the period ending with blossoming at 58 days.

The gaps in the graphs of figure 1 are the result of making measurements of dry weight of leaves from two different lots of plants which were planted a month apart. The younger plants (14 to 30 days) were planted on July third and the older ones (38 to 58 days) were planted on June second. As shown by figure 1, the plants of the later planting were growing at a more rapid rate than were the older plants, due, possibly, to more favorable environmental conditions. It is also possible, of course, that growth of maize seedlings during the first thirty days is more rapid than growth from that time on. The interesting point, however, is the fact that all three lines within a plot were increasing in dry weight of leaves at the same rate, in spite of the fact that this common rate of growth was different in the two plots. Periodic measurements of stem height, when plotted in the same manner, gave parallel curves differing as to slope in the two plots.

Degree of vascularization.—Observations, in the field, of gross size differences, together with the differences in stem and leaf measurements mentioned above, established the fact that the hybrid was significantly larger than either of the parents; but as to whether these differences were accompanied by structural differences during development necessitated a study of developmental anatomy of the stem, the most important feature of which, in the maize stem, is vascularization.

Total cross-sectional area of vascular bundles expressed as a percentage of internode cross-sectional area for each internode studied at the four stages of development is shown in figure 2. At 3 days after planting, the lower internodes of both parents showed a greater percentage of vascular tissue than did the hybrid, but the hybrid had differentiated one more internode than the more efficient parent. (Co. 2) and two more than the other parent. Vascularization in the upper internodes was a considerably greater percentage of cross-sectional area than in the basal ones. The opposite relationship existed between basal and tip internodes of the two parents. Fourteen days after planting, the hybrid continued to exhibit a higher percentage of vascularization than the parents in the tip internodes, while the

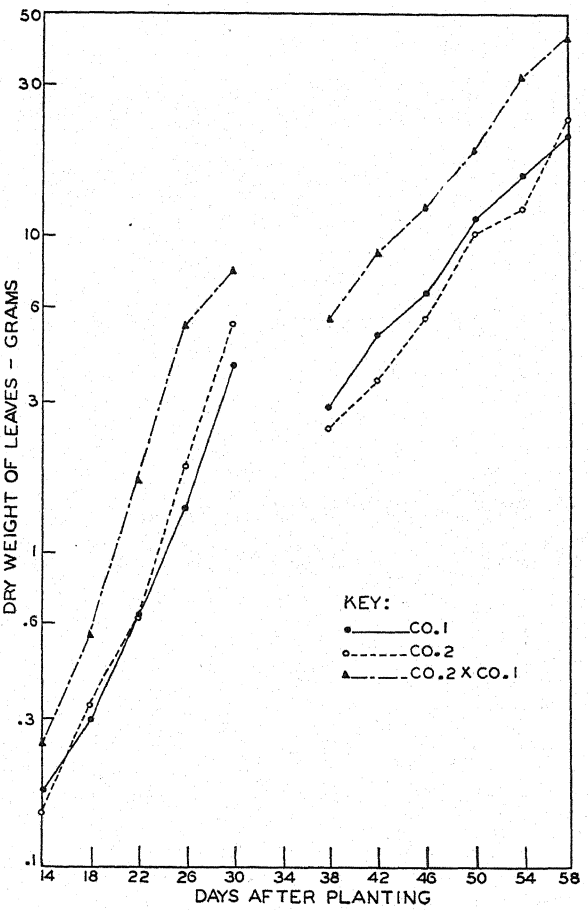


Fig. 1. Logarithm of dry weight of leaves plotted against time in days after planting. As indicated by the nearly parallel growth curves, dry weight of leaves (all leaves of a plant weighed together) increased with age at the same rate for both inbreds and the hybrid. Breaks in the curves occasioned by samplings from plantings made at different times. See "Materials and methods" and "Discussion."

internodes at the base of the hybrid had exceeded parent Co. 1 and more nearly approached the condition shown by parent Co. 2. Of the 38-day plants, the hybrid exceeded both parents in extent of vascularization in all internodes except the basal one (internode No. 7) and one internode (No. 10) of parent Co. 2. Furthermore, vascular tissue as a percentage of total internode cross-sectional area had closely approached a constant value in all internodes of the hybrid, but in the two inbred lines, the

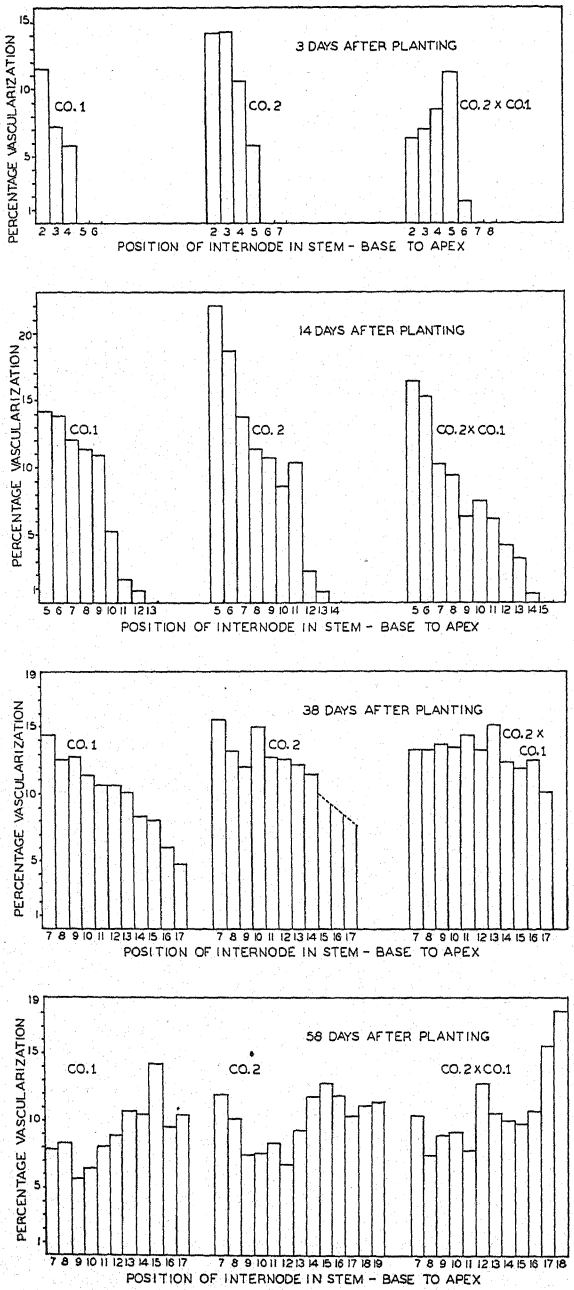


Fig. 2. Bundle cross-sectional area shown as a percentage of internode cross-sectional area for each internode of individual plants of inbreds and hybrid at four ages after planting. This figure reveals the hybrid to be less vascularized than either parent at 3 days after planting, more vascularized than parent Co. 1 at 14 days after planting, and more vascularized than either parent at 38 days and at 58 days after planting. The tip of plant Co. 2 at 38 days was lost during preparation of the slides.

value still showed a progressive decrease as the height of the internode upon the plant increased. For parent Co. 2, this decrease was less noticeable in the eight internodes studied than for the corresponding part of parent Co. 1. All internodes above the fourteenth of parent Co. 2 at 38 days after planting were lost during preparation of the slides and consequently were not available for measurements. The last samples, taken 58 days after planting, showed both inbreds and hybrids reduced in percentage of vascularization, due, presumably, to continued expansion of ground tissues after vascular bundles had reached their maximum size. The hybrid, however, exhibited a generally higher percentage of vascularization than either parent, the increase being particularly conspicuous in the topmost internodes.

In table 1 are shown further comparisons of vascularization of the two inbred parents with that of the hybrid, calculated from the percentage figures used to construct figure 2. Only those internodes common to all three plants are included in the calculations, thus giving a comparison of corresponding portions of the plants at four different ages. With the hybrid representing 100 per cent, the differences show that parent Co. 1 was exceeded in

TABLE 1. *Comparison of inbreds with hybrid as to percentage of vascularization of the entire plant. With percentage of vascularization of the hybrid representing 100 per cent, both parents are revealed to be more extensively vascularized at 3 days after planting, while the hybrid has surpassed parent Co. 1 at 14 days and is more extensively vascularized than either parent at 38 days and at 58 days after planting.*

Days after planting	Co. 2 × Co. 1 per cent	Co. 1 per cent	Difference	Co. 2 per cent	Difference
3	100.0	112.2	+12.2	173.5	+73.5
14	100.0	90.8	— 9.2	123.2	+23.2
38	100.0	82.8	—17.2	87.7	—12.3
58	100.0	87.2	—12.8	95.7	— 4.3

extent of vascularization by the hybrid at an earlier age and to a greater degree than was parent Co. 2.

Average cross-sectional area of vascular bundles. —The contribution made by vascular bundle size to greater vascularization of the hybrid was significant but not great. At three days after planting, both parents had bundles of slightly larger average cross-sectional area than those of the hybrid. Between three days and 14 days, however, the hybrid overcame this disadvantage and gained a slight advantage over both parents in average size of bundles. At 38 days after planting, the hybrid had materially increased its lead in all internodes, but the increase was greater in the lower, more nearly mature internodes than in the younger internodes above. A somewhat different relationship existed at 58 days after planting, with parent Co. 2 possessing larger bundles than the hybrid in the upper internodes and the hybrid still exceeding parent Co. 1 in all internodes and parent Co. 2 in the internodes making up the lower part of the plant. The slightly greater average size of vascular bundles in

the upper internodes of parent Co. 2 over the average for the hybrid may be the result of the more rapid rate of bundle differentiation of the hybrid. Cell division within a vascular bundle in maize stops shortly before the final steps in differentiation are completed. Presumably, therefore, cell division went on for a slightly longer period within the bundles of the inbred plants, and the one with larger bundles (Co. 2) finally exceeded the hybrid in the younger internodes. The other parent (Co. 1) had bundles considerably smaller than inbred Co. 2 throughout development and the longer period of growth apparently was not sufficient to permit that parent to equal or surpass the hybrid.

Number of vascular bundles.—Vascular bundle number accounted for the larger portion of greater percentage vascularization of the hybrid as compared with the two inbred parents. At three days after planting, more provascular strands were differentiated in all internodes of the hybrid than in the corresponding ones of the two inbred lines, the only exception being the lowermost internode of parent Co. 2. At the fourteenth day, the hybrid had increased its advantage as to number of bundles, while at 38 days, the differences between hybrid and inbreds had diminished somewhat, but the hybrid still maintained an appreciable portion of the advantage present at 14 days. In the upper internodes, parent Co. 2 possessed more bundles than the hybrid at 58 days after planting. This greater number probably was due to the fact that Co. 2 was made up of one more internode, and consequently one more leaf than the hybrid, with the additional leaf contributing its complement of vascular bundles to the internodes immediately below it.

From the studies of average cross-sectional area and number of vascular bundles, it was apparent that the greater percentage of vascularization of the hybrid, which was established sometime between three days and 14 days after planting, was effected largely by increased bundle number. This agrees with observations made by McRostie and MacLachlan (1942) on seven hybrids as compared with three standard varieties. Bundle size, however, was also operative, the combination of the two giving the hybrid the advantage which was present at 14, 38, and 58 days after planting (table 1).

Rate of differentiation.—At the three-day stage, all internodes of all three strains were still in a meristematic condition and vascularization consisted solely of provascular strands. In all three plants, the two topmost internodes were entirely undifferentiated. The plants studied 14 days after planting had only the tip internode undifferentiated, with provascular strands in successively lower internodes showing progressive stages of differentiation from strands of meristematic cells smaller and more densely cytoplasmic than those of the ground tissue to vascular bundles with well developed protoxylem and protophloem in the lowermost internode studied. At 38 days, metaphloem was almost completely differentiated and metaxylem was appearing in the bundles of the lower internodes, while the tip internodes were still in the provascular-strand stage, and at 58 days, vascular bundles were completely differentiated in all internodes from the base to a point approximately half way up the stem. Above that point, differentiation was increasingly less complete until, in the tip internodes, the vascular strands consisted of metaphloem and protoxylem with metaxylem in an early stage of development.

While differentiation of vascular bundles was at approximately the same stage in comparable internodes of both parents and the hybrid, careful microscopic examination of the most nearly mature bundles of each internode at each of the four ages revealed that the hybrid, Co. 2 × Co. 1, was generally more completely differentiated at comparable levels on the stem than was either parent. Inbred Co. 2 was differentiated to a slightly greater degree than was inbred Co. 1. At 58 days after planting, the differences between Co. 2 and Co. 2 × Co. 1 were less pronounced at all levels than at the 38-day and 14-day ages, indicating that for a certain period the hybrid had maintained a greater rate of differentiation of vascular tissue but that sometime between 38 days and 58 days after planting this increased rate had diminished. In parent Co. 2, the more efficient of the two inbred strains, the rate during the same period had been maintained at more nearly the maximum level, resulting in a close approach by Co. 2 to the level of differentiation attained by the hybrid. As criteria of degree of differentiation, the vascular bundles were compared as to stage of development of protoxylem and protophloem, the time of appearance and stages of development of metaxylem, and time of appearance and stages of development of metaphloem. Since both metaxylem and sieve-tube cells start out as small, densely cytoplasmic cells within the bundle and steadily increase to a definite size at maturity, comparative sizes were made use of in contrasting the three lines as to degree of differentiation. Amount of lignification, as revealed by intensity of staining with safranin, was also studied, as was the time of disappearance of nuclei and cytoplasm from the metaxylem cells.

Cell size.—Averages of the cross-sectional areas of twenty medulla cells at three levels (base, midway of the stem, and tip) on each plant at each of the four ages were computed. Comparisons of hybrid with parents at corresponding levels and ages revealed that there was no constant relationship as to cell size between the three lines. At some levels at the different ages, medulla cells of the hybrid were larger than those of either parent, while at other levels they were intermediate between or somewhat smaller than those of the parents.

Metaxylem cells, on the other hand, showed a greater average cross-sectional area in the hybrid during development, but at maturity both parents and hybrid possessed metaxylem cells of approximately the same size. At 38 days after planting, the earliest age at which metaxylem cells were suffi-

ciently differentiated to permit study, those of the hybrid were significantly larger than in either parent, were differentiated to a greater degree, and were differentiated in more internodes of the plant. Metaxylem was differentiated in the eleventh internode of the hybrid plant at 38 days but had not reached a comparable stage above the eighth internode in either parent, a fact which may be of significance as contributing to the greater vigor of the hybrid, but which, conversely, may be merely a result of differentiation keeping pace with the increased size of the hybrid. At 58 days after planting, metaxylem cells were about the same size as those of parent Co. 2 in the basal internodes, and intermediate between those of the two parents in the internodes above number eleven.

A comparable condition existed with regard to sizes of sieve-tube cells at the different ages. At 14 days after planting, sieve-tube cells of the hybrid were smaller in cross-sectional area than those of either parent. By the end of 38 days they were larger than those of either parent in all internodes and were differentiated in all internodes of the plant. In parent Co. 1 no differentiation of phloem had taken place above internode 13 nor presumably above internode 14 in parent Co. 2, although internodes above the fourteenth of that line were not available for measurement. Parent Co. 2 had approximately equaled the hybrid in size of sieve-tube cells in all internodes up to the eleventh at 58 days after planting; while above that point the hybrid was intermediate between the two parents.

Discussion.—Size differences such as those exhibited by the first-generation hybrid plants studied were perhaps to be expected since Murdoch (1940) reported that the embryos from seeds of the cross Co. 2 × Co. 1 were significantly heavier than those of either Co. 1 or Co. 2, and Copeland (1940) has stated that hybrid maize zygotes exhibit hybrid vigor as early as four days after fertilization. Consequently, greater size of the hybrid plants should result from the size advantage established before maturity of the embryo if subsequent growth went on at the same or a greater rate.

It is noteworthy that the advantage possessed by the hybrid at the earliest age studied was maintained at a constant rate during development. Constant rates of growth of stem and leaves of hybrid and inbreds are made even more striking by the fact that the plants that were measured for leaf mass and stem height were planted at two different times, 30 days apart, and grew at two distinctly different rates in the two plots. However, the growth rates were the same for all three strains within a plot, which suggests that both parents and hybrid reacted in the same manner to environmental factors such as length of day, relative humidity, soil moisture, and soil fertility. These data are in agreement with results of Ashby (1937) and Luckwill (1939) showing that greater size of hybrids was not the result of more rapid growth after germination, but was due to maintenance of an initial advantage in embryo size. Studies reported by others of vigorous hybrids of maize and other plants, however, indicate that hybrid vigor cannot always be attributed to greater embryo size resulting from a greater rate of development of the zygote. Thus Hatcher (1940) and other workers report cases of extreme vigor exhibited by hybrid plants from embryos smaller than, or intermediate in size between, those of the parents. In such cases, it is obvious that growth rate must be greater in the hybrid at some time between germination and maturity.

The present investigation of internal structure of inbred and hybrid plants during development brings out the fact that, unlike the rate of over-all size increase, rate of development of bundles and final extent of vascularization were not the same in hybrid and inbreds. Vascularization of the hybrid maize stem was not merely maintenance of an initial advantage. The hybrid was actually at a disadvantage in ratio of vascular tissue to total area in the beginning, while later it was more extensively vascularized than either parent. It seems evident, then, that one important manifestation of hybrid vigor may be the greater percentage of vascularization of hybrid plants. It is possible, nevertheless, that the increased percentage of vascular tissue in the larger plants may be merely a normal manifestation of increased stem size, perhaps related to the tendency stressed by Bower (1930) for relative increase in vascular surface in larger axes, made necessary by the fact that stem volume increases as the cube of its linear dimension while vascular surface increases only as the square.

It may be suggested, also, that more extensive vascularization of the hybrid may be closely associated with the more rapid rate of differentiation. Initiation of vascular tissue is clearly one aspect of differentiation in the plant as a whole, and it is possible that the laying down of provascular strands within the ground tissue and differentiation of the specialized tissues within those strands both are manifestations of the same physiological differences between hybrid and inbred.

The very fact of accelerated rate of differentiation seems to be an important physiological distinction between hybrids and inbreds and, although the nature of the mechanism resulting in this difference cannot be demonstrated from the results of this investigation, it seems that maintenance of this higher rate of differentiation within developing bundles and the fact that provescular strands were initiated in younger internodes of the hybrid than in the inbreds, together with the greater extent of vascularization of the hybrid, constitute manifestations of hybrid vigor entirely separate from over-all size differences.

Developmental analysis of inbred and hybrid maize has made it possible, therefore, to distinguish between internal manifestations of hybrid vigor and the more commonly observed increase in over-all size of hybrids as compared with their inbred parents, and has shown these phenomena to

be distinct from each other. Furthermore, from the study it has become evident that an explanation of the phenomenon of heterosis must account for the greater relative vascularization of hybrid plants and for the more rapid rate of differentiation within them.

SUMMARY

Two maize inbreds and their first-generation hybrid were studied to determine whether histological differences existed, during development, between the parents and the more vigorous hybrid. Studies were made of stem heights, length and cross-sectional area of individual internodes, total dry weights of leaves, ratio of leaf length to leaf width, ratio of vascular cross-sectional area to internode cross-sectional area, number and average cross-sectional area of vascular bundles, and sizes of medulla, metaxylem and sieve-tube cells at various ages during development of the plants.

The investigation brought out the following points in connection with development of the maize plants studied: (a) At maturity, average cross-sectional area of internodes studied was the same in all internodes below the thirteenth. Above that, a progressive decrease in size was shown in successive internodes toward the apex. (b) At maturity, in all internodes studied, bundle number was the same in all internodes below the thirteenth, and decreased progressively toward the apex.

The hybrid seeds germinated earlier and hybrid plants were taller than the parents and possessed a greater volume of leaves throughout development. Hybridization did not alter the rates of increase in stem height, internode length, internode cross-sectional area, volume of leaves, nor ratio of leaf length to leaf width. At the first sampling, both parents were more extensively vascularized than the hybrid, but the hybrid soon gained an advantage and at all other ages studied showed a greater percentage of vascularization than either parent. The hybrid exhibited an accelerated rate of differentiation, with provascular strands present in younger internodes than in either parent and with differentiation of conducting tissues within the bundles more nearly complete in the hybrid than at comparable levels in the inbred plants.

Developmental analysis of hybrid and inbred maize has shown that attainment of greater size by hybrids is a process distinct from that controlling differentiation. This study indicates that any explanation of heterosis must account for differences between hybrid and parents in relative vascularization and rate of differentiation.

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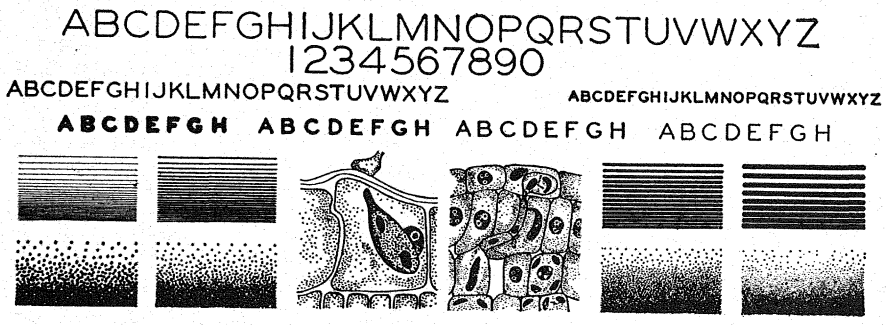
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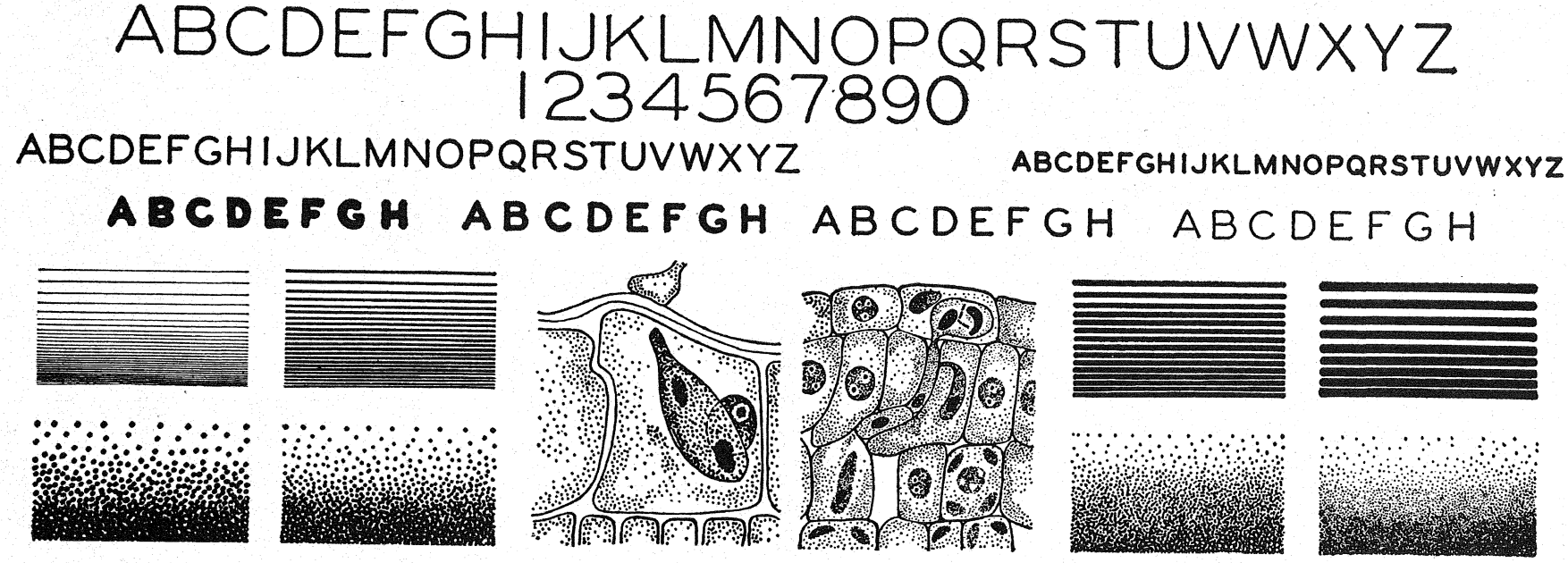
DRAWING GUIDE FOR ZINC ETCHINGS

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THE NATURE OF SEXUALITY IN CHLAMYDOMONAS [1]

Gilbert M. Smith

In 1933 Moewus published the first of an extensive series of articles on the nature of sexuality in, and the genetics of, freshwater algae. The algae he has studied include *Chlamydomonas, Polytoma, Stephanosphaera, Protosiphon, Monostroma,* and *Botrydium.* There has been widespread interest in the work of Moewus, especially that on *Chlamydomonas,* and the results he has reported have evoked considerable debate. This discussion will be limited to the series of Moewus' investigations centering around the nature of the substances that induce motility and fusion of gametes in *Chlamydomonas.* A brief preliminary report will also be given on sexuality in some species of *Chlamydomonas* collected in California.

Moewus isolated *Chlamydomonas* in unialgal and pure culture by means of the well-known agar plate method. As is familiar to all who have grown this alga it forms an immobile green jelly-like mass or Palmella stage when growing on the surface of agar, but the cells soon become motile if the culture is flooded with water. The species studied by Moewus were of the type in which every motile cell is a potential gamete and capable of fusing with any cell of opposite sex swimming in its immediate vicinity. In heterothallic (heteroecious) species one must have two cultures of opposite sex and mix swarmers of the two before there is gametic union. In homothallic (synoecious) species, gametic union is between cells of the same culture, but does not take place until after the cells have become motile.

When studying heterothallic species Moewus uses the method devised by Hartmann (1929) for observing gametic union. Two drops of water, one containing male gametes and the other containing female gametes, are placed side by side on a slide without a cover glass. After focusing under the microscope the two drops are mixed together. Within five seconds there is a very marked aggregation of the gametes in clumps. Up to a certain concentration of gametes the size of clumps after mixing the two kinds is correlated with the concentration, but with an increase beyond ten million gametes per cubic centimeter there is no further increase in number of gametes in a clump. The gametes in each clump soon begin to fuse in pairs and when two begin to fuse there is a reversal of phototaxis and a migration of the fusing pair to the shaded side of the drop. This reversal of phototaxis, first noted by Carter (1926) in *Monostroma,* makes it easy to find fusing pairs of gametes when those of opposite sex are mixed.

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When Moewus (1933) placed palmelloid cultures in darkness for 24 hours and then flooded with water the cells remained immobile, but if he flooded the culture with a 1 per cent glucose solution the cells became motile. In addition to glucose there are several other sugars that induce motility in darkness (Moewus, 1939a) but with none of these sugars, as with glucose, is there any gametic union after the cells become motile. When palmelloid cultures in darkness are illuminated with visible light of any wave length between 4358 and 6430 Å the gametes become motile. However, they are only capable of uniting in pairs when illuminated by light from the blue end of the spectrum. To be more specific, Moewus (1939a, p. 486) finds that there is gametic union in light of 4358 and 4961 Å, but no union in light of 5361, 5770, 5791, 5890, or 6430 Å.

Moewus flooded palmelloid cultures that had been standing in light and after the gametes had become motile removed all gametes by filtering or centrifuging. When palmelloid cultures in darkness were flooded with these filtrates or centrifugates the cells became motile and sexually functional. In heterothallic species female cultures were made sexually functional in darkness by filtrates from female cultures in light, but not by filtrates from male cultures in light. The reverse is true for male cultures (Moewus, 1939, p. 43). However, a filtrate from gametes in light is not without effect upon gametes of the opposite sex. If female gametes swimming in light are placed in a filtrate from male gametes swimming in light, or vice versa, the gametes group in clumps but the individuals do not fuse in pairs. For some unexplained reason this only takes place in cultures contaminated with bacteria (Moewus, 1933).

Moewus obtained further insight into the nature of substances excreted by gametes by studying the effect of light on filtrates from *C. eugametos.* When female gametes were made motile in darkness by means of a glucose solution and the filtrate exposed to daylight the filtrate had no effect on sexuality of motile cells in darkness. On the other hand, a filtrate from gametes swimming in red light does contain the precursor (V) of a substance causing gametic union (Moewus, 1938). After the filtrate containing the precursor from female gametes has been exposed to blue light for 10–30 minutes it contains a substance capable of making female gametes functional in darkness. This female copulation substance (K ♀) disappears after illumination in blue light for more than 30 minutes, but if illumination is continued for 75–90 minutes the filtrate contains a substance capable of making male gametes functional in darkness. The male copula-

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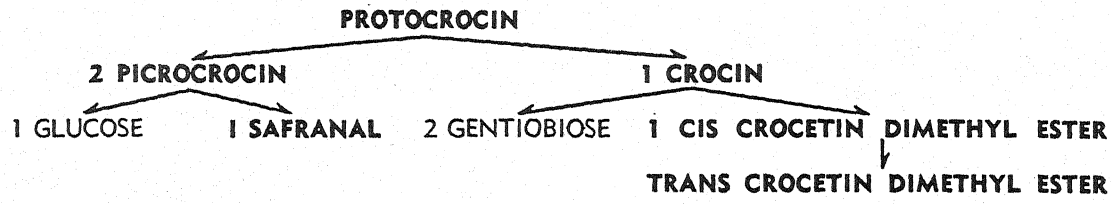


Fig. 1. Diagram showing the carotinoids and sugars resulting from the breakdown of protocrocin. (From Moewus, 1940.)

tion substance ($K\,\delta$) disappears 90 minutes after illumination in blue light and with further illumination neither the female nor the male substance reappear in the filtrate. Illumination for more than 90 minutes eventually results in an end substance (K_O). If filtrates from male gametes swimming in red light are exposed to blue light there is no formation of the female copulation substance, but there is a formation of the male copulation substance and the end substance. When written as formulae the series of changes may be expressed as follows:

Female filtrate ..... $V \rightarrow K♀ \rightarrow K♂ \rightarrow K_O$
Male filtrate ..... $V \rightarrow K♂ \rightarrow K_O$

Moewus also finds that he can obtain the male and female copulation substances by mixing the precursor and the end substance in definite proportions. This was first done with *C. eugametos*. In this species a mixture of three parts precursor and one part end substance produces the female copulation substance. A mixture of one part precursor and three parts of end substance produces the male copulation substance (Moewus, 1938). Neither male nor female substances of *C. eugametos* are produced in mixtures of 1:1, 2:1, 4:1 or other proportions.

Thanks to the efforts of the biochemist Kuhn, who became interested in the work of Moewus, the chemical nature of the sexual substances has been determined. The sexual substances were concentrated from 200 l. of a rich filtrate but the amounts obtained were so small that determination of their nature had to be based upon spectroscopic analysis instead of direct chemical analysis. Some idea of the minute amount obtained may be gained from the estimate that there is but 1 mg. in 1000 l. of a rich filtrate (Kuhn, Moewus, and Wendt, 1939). On the basis of spectroscopic analysis the sexual substances were found to be carotinoids. It is held that the first-formed substance is protocrocin. This breaks down into two molecules of picrocrocin and one of crocin (fig. 1). Each molecule of picrocrocin breaks down into a molecule of glucose and a molecule of safranol. Each molecule of crocin breaks down into two molecules of gentiobiose and one molecule of cis crocetin dimethyl ester which, in time, becomes trans crocetin dimethyl ester. Genetic analysis has shown (Kuhn and Moewus, 1940, Moewus, 1940a) that each step in the degredation of protocrocin is brought about by a different gene.

By the addition of each of the foregoing carotinoids to the appropriate filtrate it was found that each plays a specific role in gametic union. Crocin, or a very closely related glucoside of crocetin, is the chief substance inducing motility in gametes. For *C. eugametos* it is effective in a concentration of 1:250 trillion. From this it has been computed that a crocin solution with one or a very few molecules per cell will make them motile (Kuhn, Moewus, and Jerchel, 1938, Moewus, 1938). The necessary concentration of crocin has been studied in two other species and found to be 100,000 molecules per cell for *C. dresdenensis* and 1,000,000 per cell for *C. Braunii* (Moewus, 1939a).

The cis and trans crocetin dimethyl esters are primarily concerned with the mutual attraction of gametes (fig. 2). Moewus (1940) calls these attracting substances gamones, but it should be noted

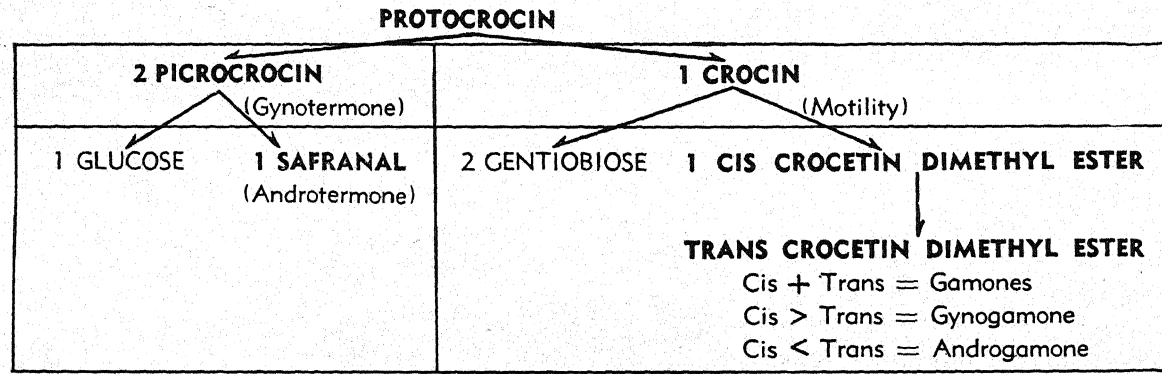


Fig. 2. Diagram showing the function of the various carotinoids resulting from the breakdown of protocrocin. (Based on Moewus, 1940.)

that he uses the term in a somewhat different sense from Hartmann and Schartau (1939) who coined the term. Gamones are neither pure cis nor pure trans crocetin dimethyl ester but a mixture of the two. The gamone (gynogamone) of a female gamete contains more cis than trans crocetin dimethyl ester: the reverse is the case with the gamone (androgamone) of a male gamete. The substances that Moewus called the precursor (V) and end substance (K_O) are, respectively, pure cis and pure trans crocetin dimethyl ester.

Picrocrocin and safranol are sex determining substances and have been called termones (Kuhn, Moewus, and Wendt, 1939). Picrocrocin is the termone (gynotermone) of female gametes and safranol is the termone (androtermone) of male gametes.

Another phase of Moewus' work is concerned with the intensity of maleness and femaleness from species to species. There are certain algae where he finds the difference in intensity so pronounced that a female gamete of one species will unite with a female gamete of another species. The same holds for male gametes of the two. He has studied this phenomenon of relative sexuality most thoroughly in *Chlamydomonas*. Four intensities of maleness and femaleness were first reported for this genus (Moewus, 1939), later a fifth intensity was discovered (Moewus, 1940a). Moewus calls the intensity of sexuality the valence and numbers the valences from 1 to 5 according to their increased intensity. The inter-relationships between the different valences were studied at a time when only valences 1 to 4 were known. These inter-relationships were shown by making all possible combinations between male and female gametes of four interfertile heterothallic strains, each of a different valence. In this series the greatest difference in intensity is between male and female gametes of valence 4. Here the total difference is 9. The difference in intensity is less for other combinations and is 0 as between male (or female) gametes of the same valence. Although there are nine differences in intensity in this series there are but four types of reaction when gametes are brought together under optimum conditions. These four types of reaction are: no fusion of gametes (Reaction 0), isolated pairs of fusing gametes throughout the mixture (Reaction 1), clumps of 10–20 gametes (Reaction 2), and clumps of 100 or more gametes (Reaction 3).

The relationship between the different types of reaction becomes evident when the results from the various combinations are arranged in a checkerboard diagram (fig. 3). Such a diagram shows that the type of reaction depends upon the total difference in intensity of sexuality in the two kinds of gametes in a mixture. If there is no difference or if the total difference is 1 there is no gametic union (Reaction 0). When the total difference is 2 there are isolated pairs of fusing gametes (Reaction 1) and when the total difference is 3 there are clumps of 10–20 gametes (Reaction 2). A total difference of 4 or greater produces clumps of 100 or more gametes (Reaction 3). The fact that gametic union may take place when the difference in intensity is as low as 2 shows why there may be a fusion of gametes of the same sex in interfertile species. Increase of vigor of reaction with increase in difference in total intensity shows why the type of reaction is not the same for all cases of fusion between gametes of the same sex.

By illumination of filtrates with blue light for different lengths of time, and by using various mixtures of cis and trans crocetin dimethyl ester, Moewus (1940a) has shown that valence is due to the relative proportions of cis and trans crocetin dimethyl ester. Gametes of the highest valence have the most pronounced difference of the two, and the differences between the two are smaller for gametes of lower valence. The proportion of cis and trans crocetin dimethyl ester are sharply delimited for each valence and do not vary by more than two per cent from the ratios given in figure 4.

	♀4	♀3	♀2	♀1	♂1	♂2	♂3	♂4
♀4	0	0	1	2	3	3	3	3
♀3	0	0	0	1	3	3	3	3
♀2	1	0	0	0	3	3	3	3
♀1	2	1	0	0	2	3	3	3
♂1	3	3	3	2	0	0	1	2
♂2	3	3	3	3	0	0	0	1
♂3	3	3	3	3	1	0	0	0
♂4	3	3	3	3	2	1	0	0

Fig. 3. Table showing the type of reaction (0, 1, 2, or 3) between gametes of different valence when of the same or different sex. For a description of reactions 0, 1, 2, and 3 see text. (From Moewus, 1939.)

The demonstration that valence is due to the cis/trans ratio affords a more precise means of distinguishing between differences in valence and the resultant fusion or non-fusion of gametes. In any two different gametes both the cis and the trans crocetin dimethyl esters differ by the same amount and by making proper combinations between valences 1 to 4 it is possible to go by ten point steps from a ratio of 0/0 to a ratio of 90/90. In this series of steps the type of reaction is dependent upon the cis/trans ratio (fig. 5). There is no gametic union if the ratio is 0/0 or 10/10. A

ratio of 20/20 results in the type 1 reaction, a ratio of 30/30 gives the type 2 reaction, and a difference of 40/40 or greater produces the type 3 reaction. Fusion between gametes of the same sex takes place in those cases where the difference is 20/20 or 30/30.

One can see from the foregoing account why the work of Moewus has aroused so much interest. One of the reasons why others have not worked along similar lines becomes apparent when one begins the tedious task of trying to isolate strains of *Chlamydomonas* that regularly reproduce sexually. Moewus states (1940, p. 420) that he spent three years isolating material from more than five hundred samples of soil in obtaining an interfertile group of species. The interfertility of the five species with which he worked, the so-called *eugametos* group, shows that they are related. On the other hand, if one follows the practice of those (Pascher, 1927; Gerloff, 1940) who divide the genus into six subgenera these five species belong to three different subgenera. One species (*C. eugametos*) belongs to the subgenus *Chlamydella;* two (*C. oögama* and *C. paupera*) belong to the subgenus *Chloromonas;* and two (*C. dresdenensis* and *C. Braunii*) to the subgenus *Euchlamydomonas*. Even if these subgenera are artificial and thus exaggerate differences it is obvious that these five interfertile species differ markedly in morphological characters.

Some argue that one must have members of the *eugametos* group before attempting to work in this field. Since Moewus records (1940b, 1940c) forms of *C. eugametos* from soil from various parts of the world it was hoped that one or more of them would be present in samples of soil collected in California. Thus far this hope has not been realized and none of the sexual strains isolated from Californian soil belong to the *eugametos* group. Even though members of the *eugametos* group have not been at hand, it has been thought worth while to study the sexuality of the Californian material and to compare the sexuality of these species with that recorded for the *eugametos* group.

All isolation of material from California was by the agar plate method and the species isolated were cultured in an immobile palmelloid condition on agar. The sexuality of the various isolates was studied after the cultures had been changed from a palmelloid to a motile condition by flooding with Beyerinck's nutrient solution.

Practically all of the isolations showing sexuality appeared to be homothallic when first tested. All isolations showing sexuality were replated immediately after the cells had become motile after flooding and twenty clones were reisolated from each. Of the four species regularly showing gametic union after flooding, three proved to be heterothallic when reisolated and the fourth homothallic. It is well known for heterothallic species of *Chlamydomonas* that half the cells produced by a germinating zygote are male and half are female. If all the cells from a germinating zygote of a heterothallic species were to grow into a single palmelloid colony this would produce both male and female gametes. It is thought that this is the explanation for the homothallism of the original isolations and the heterothallism of the reisolations. That is, the colony appearing in the original plating came from a zygote instead of from a vegetative cell. Such cultures have one distinct advantage: there is no doubt as to whether or not reisolations of opposite sex are the same species.

Every phycologist knows that accurate identification of a majority of the species of *Chlamydomonas* is an exceedingly difficult matter. For this reason, determination of the sexual species collected in California must be accepted with reservations. The heterothallic species identified as *C. intermedia* Chodat was isolated from several samples of soil collected in a paddock formerly used for horses of the R.O.T.C. unit at Stanford University. The heterothallic species identified as *C. minutissima* Korschik. was isolated from soil collected in an orchard near Mountain View and an orchard south of San Jose. Cells of a heterothallic strain isolated from soil from March Field near Riverside, California, superficially resemble *C. minutissima,* but produce a type of clump not previously described. Here the gametes become arranged in a star-shaped cluster with their anterior ends pointing towards the center of the cluster. It might be thought that this unusual type of cluster is due to grouping of many male gametes about a single female gamete. Continuous observation of a single cluster shows that this is not the case because more than one pair of fusing gametes can be seen emerging from the clump. The homothallic strain identified as *C. Snowiae* Printz was isolated from soil collected at Pacific Grove. A very distinctive species, *C. stellata* Dill, has been found in two samples of soil from Pacific Grove, but reproduction in all isolations of it is exclusively asexual.

Moewus repeatedly states that all members of the *eugametos* group do not become motile in darkness when flooded with water or with inorganic salt solutions. When palmelloid cultures of all five spe-

VALENCE	♀5	♀4	♀3	♀2	♀1	♂1	♂2	♂3	♂4	♂5
CIS/TRANS RATIO	98.2/1.8	95/5	85/15	75/25	65/35	35/65	25/75	15/85	5/95	1.8/98.2

Fig. 4. Table showing the proportions of cis and trans crocetin dimethyl esters in male and female gametes of different valence. (From Moewus, 1940a.)

cies from California were placed in the dark room for 24 hours and then flooded with Beyerinck's solution the cells became motile. This was shown by disappearance of the green coating from the surface of the agar and the uniform green color of the water. Motility of the cells was confirmed by microscopical examination within 10 seconds after a sample had been removed from the dark room. There is the possibility that the motility might have been due to the short time a red light was turned on in the dark room while the cultures were being flooded. To show that this is not the case, palmelloid cultures were placed on a table where they could be found by feeling around in total darkness. When found and flooded in darkness by this "touch system" the cells became motile.

In another experiment seven cultures of each species were placed in the dark room and one culture of each species flooded every 24 hours. On every day of the seven the cells of each species became motile when flooded in darkness. When Moewus transferred motile cells from light to darkness the motility did not persist for more than an hour (Moewus, 1938; p. 759). From this one may conclude that crocetin, the motility-inducing substance of the *eugametos* group, does not remain effective for more than an hour after it is formed in light. If the motility-inducing substance of the Californian species of *Chlamydomonas* is crocetin, its persistence in darkness for more than a week is very remarkable.

All three heterothallic species collected in California also have a union of gametes in darkness. This is shown by placing two palmelloid cultures of opposite sex in the dark room for one, two or more days and then mixing the two after motility has been induced by flooding in darkness. The best method for proving gametic union in darkness is to allow the mixture of motile gametes to remain in the dark room for 15 minutes and then to kill all swarmers by adding a few drops of an iodine potassium iodide solution to the mixture before taking to the laboratory. When mixtures treated in this manner are examined microscopically 20 to 50 per cent of the swarmers are quadriflagellate zygotes in various stages of fusion.

The fact that gametes of the Californian material are sexually functional in darkness has precluded use of Moewus' most effective tool—the analysis of sexuality by means of illuminating filtrates from gametes. This did not prevent repeating his experiments on the effect of a filtrate from one sex upon gametes of the opposite sex. When Moewus (1933) did this with *Chlamydomonas eugametos,* or *Protosiphon botryoides,* or *Stephanosphaera pluvialis* he found that filtrates from the opposite sex produced a clumping, but no fusion, of gametes of the opposite sex—provided bacteria were present. He prepared his filtrates by filtering through a Schott 3 on 5 frittered glass filter and this was done in red light to avoid breakdown of the sexual substances. Filtrates from all three heterothallic species collected in California were also prepared in red light by means of a Schott 3 on 5 filter, and the same type of bacteria added, but all results were negative. No comment will be made because it is impossible to prove beyond all doubt that the failure to duplicate Moewus' results was not due to faulty technique.

Thus far everything reported about the three heterothallic species collected in California is a negation of what Moewus reports for the *eugametos* group. On the other hand, there is evidence showing that gametic union in the Californian mate-

	95/5 ♀4	85/15 ♀3	75/25 ♀2	65/35 ♀1	35/65 ♂1	25/75 ♂2	15/85 ♂3	5/95 ♂4
95/5 ♀4	0/0 0	10/10 0	20/20 1	30/30 2	60/60 3	70/70 3	80/80 3	90/90 3
85/15 ♀3	10/10 0	0/0 0	10/10 0	20/20 1	50/50 3	60/60 3	70/70 3	80/80 3
75/25 ♀2	20/20 1	10/10 0	0/0 0	10/10 0	40/40 3	50/50 3	60/60 3	70/70 3
65/35 ♀1	30/30 2	20/20 1	10/10 0	0/0 0	30/30 2	40/40 3	50/50 3	60/60 3
35/65 ♂1	60/60 3	50/50 3	40/40 3	30/30 2	0/0 0	10/10 0	20/20 1	30/30 2
25/75 ♂2	70/70 3	60/60 3	50/50 3	40/40 3	10/10 0	0/0 0	10/10 0	20/20 1
15/85 ♂3	80/80 3	70/70 3	60/60 3	50/50 3	20/20 1	10/10 0	0/0 0	10/10 0
5/95 ♂4	90/90 3	80/80 3	70/70 3	60/60 3	30/30 2	20/20 1	10/10 0	0/0 0

Fig. 5. Table showing the relationship between type of reaction between gametes and differences in amounts of both cis and trans dimethyl esters in the gametes. (Based on Moewus, 1939.)

rial is more intense in light than in darkness. When gametes swarming in darkness are taken to light and immediately mixed there are isolated fusing pairs in the mixture. If the two are mixed a minute after transfer to light there are usually clumps of 4–5 gametes and if they are not mixed until after they have been standing in light for 3–5 minutes there is a formation of clumps of 8–20 gametes. The accelerating effect of light on gametic union was also shown in another way. Two cultures of motile gametes of opposite sex were mixed in the dark room. The mixture was divided into two portions, one of which was taken to light. The portion taken to light was gently swirled to preclude phototactic accumulation and thus a difference in concentration of gametes in the two lots. At the end of 5 or more minutes both lots were killed and the relative number of gametes and quadriflagellate zygotes noted. Usually the greater number of zygotes in the portion standing in light is obvious. Random counts of 100 swarmers in each of the two lots shows this

even more clearly. For example, a random count of 100 swarmers of *C. minutissima* gave 31 gametes and 69 zygotes for the dish in light as against 71 gametes and 29 zygotes for the dish in the dark room.

The results obtained in this preliminary inspection of sexuality as found in three species collected in California are so incomplete that detailed comparisons with the results recorded by Moewus are not justified. However, sufficient progress has been made to show that the sharp correlation between illumination and development of sexuality reported for the *eugametos* group does not hold for the genus as a whole. The question as to whether or not the sexual substances of species collected in California are fundamentally the same as those of the *eugametos* group remains one for further investigation.

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INTERSPECIFIC CROSSES INVOLVING DATURA CERATOCAULA OBTAINED BY EMBRYO DISSECTION [1]

Susanne W. McLean

The genus of herbaceous Daturas is made up of ten species which are widely distributed throughout the world. All of them, with the exception of *D. ceratocaula,* are dichotomous, produce spiny capsules and are adapted to relatively dry habitats. *D. ceratocaula,* a species endemic to Mexico, has whorled branching, smooth capsules and grows in shallow ponds. It has modifications, such as a hollow stem and a reduced amount of vascular tissue, which suit it for this type of environment.

In the Datura work it was found important to secure hybrids between the various species. However, though numerous attempts were made, no hybrids were ever produced between *D. ceratocaula* and any of the other species. This is not surprising considering the great morphological differences existing between them. A few years ago the embryo dissection technique was introduced into Datura research as a method for obtaining hybrids from incompatible crosses. A number of the species crosses in Datura which did not produce viable seeds were found by Blakeslee and Satina (1944) to yield arrested embryos which could be cultured in nutrient media and grown to maturity. With this in mind it was decided to make a study of the crossability of *D. ceratocaula* with the other nine species. This study was begun with the race of *D. ceratocaula* in Dr. Blakeslee's collection (race I).

During the course of the research a new race of *D. ceratocaula* (race II) was obtained from seeds collected by W. G. Whaley in Villa Madero, Du-

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rango, Mexico. It was found to differ somewhat in chromosomal structure from race I (Jean Cummings, unpublished). Certain evident morphological differences can be listed. (1) Flowers of race II are much smaller than those of race I; (2) race II has broader leaves; (3) buds of race I remain erect throughout development, whereas race II buds are pendant from the time they are about one centimeter in length until just before flowering; (4) apparently, under greenhouse conditions at least, race II has no tendency toward whorled branching; and (5) race II has much less purple pigment as shown by the pale corolla and by the lack of bright purple pigment in the inner capsule wall which is characteristic of race I. Only race I is considered in this paper.

MATERIALS AND METHODS.—The plants were from Dr. Blakeslee's collection of herbaceous Daturas from many parts of the world. Frequently different chromosomal types of the same species were used. The plants usually were grown under greenhouse conditions, although in the summer time a few were grown in the field. The work was carried on over a period of two years, from the summer of 1943 through the summer of 1945.

The desired cross-pollinations were made both in the greenhouse and in the field. No attempt to prevent pollination by insects was necessary in the screened greenhouse, but in the field the following procedure used with Daturas was employed. After pollination a section of a soda fountain straw, closed at one end with sealing wax, was placed over the stigma and style. The corolla was tied around the straw to keep it in place. In making crosses the pollen was frequently saved for several days, though usually not longer than four or five.

Twenty to thirty days after pollination the capsules were collected. Dissection methods followed those of van Overbeek, Conklin, and Blakeslee (1942). The capsules were sterilized by dipping them in alcohol. The seeds were removed and opened under sterile conditions. The embryos were taken from the seeds under a binocular microscope and placed in small cotton-plugged vials (5.0 cm. by 1.0 cm.) containing 1.0 cc. of nutrient which were then kept in a dark incubator at a temperature around 26°C. The embryos were transferred to fresh nutrient approximately every two weeks. The composition of the nutrient varied considerably over the course of the experiment, but in general it contained inorganic salts and sucrose with or without agar. The concentration of sucrose was usually 2.0 per cent, and the inorganic salt formula either was a modification of that of Tukey (1938) or was that used by Randolph and Cox (1943). For the growth of young embryos 0.5 per cent malt extract was added (Blakeslee and Satina, 1944). The cultures were left in the incubator until they were around a centimeter in length when they were brought into diffused light. The time varied anywhere from a few weeks to several months. As soon as the young seedlings began to produce roots they were placed in larger cotton-plugged vials (about 7.0 cm. by 1.5 cm.) where they were left until ready to be planted in soil. If the nutrient began to dry out, sterile distilled water was added. Care was taken not to transfer a seedling more frequently than necessary because of the danger of breaking the delicate roots.

When the roots were growing vigorously the seedlings were transplanted to a sandy soil. At one time the potted plants were kept in the laboratory until they had established themselves, but later it was found far better to put them immediately into the greenhouse where the temperature and humidity were much more favorable.

Among the cultures, plants were often found having shoots without roots, or roots without shoots, or neither. For the first case a very valuable technique was available (Blakeslee, 1945). A micrograft of the shoot was made onto a young, vigorous plant of *D. stramonium*. This was covered by a gelatin capsule, the edges of which were paraffined to keep it airtight. The capsule was removed when the graft had become secure.

SEED GERMINATION OF D. CERATOCAULA.—Under greenhouse conditions approximately 0.1 per cent of the seeds of *D. ceratocaula* germinate. It was found (Lela V. Barton, unpublished) that this extremely low percentage of germination could be raised considerably by stratifying the seeds over a period of several months at low temperatures. However, this method led to variability in individual plantings and so was not dependable. The writer found that a high germination percentage could be secured by use of the embryo culture technique in which the embryos are excised from the seed and grown in artificial nutrient. By this method an average germination of 60–80 per cent was obtained.

Such an increase in germination by use of the technique of embryo culture (in which the embryo is freed from the rest of the seed) led to the belief that perhaps parts of the seed coat contained some factor or factors inhibiting growth such as has been found with certain other seeds. The following experiment was set up to test this hypothesis. The seed coat of *D. ceratocaula* is made up of a hard outer layer and an inner softer one in which the embryo is imbedded. This inner layer may be compound in nature. Here it is simply referred to as the inner layer of the seed coat. Embryos with and without parts of the seed coat were cultured in a nutrient suitable for mature embryos. Six groups with eleven to twelve embryos each were set up. These were as follows: (A) whole seeds; (B) seeds with approximately one-half of the outer layer of the seed coat removed; (C) seeds with entire outer layer removed; (D) seeds with approximately one-half of the outer and one-half of the inner layer removed; (E) seeds with the entire outer layer and approximately one-half the inner layer of the seed coat removed; (F) embryo only. Groups (D) and (E) obviously offered some difficulties in dissection. An attempt was made to slice off one side of the seed as far as the embryo. Sometimes a very thin

layer of tissue was left over part of the embryo and sometimes a bit of the embryo was sliced off. However, this latter injury has been found to have little or no effect on the growth of excised embryos. It was thought the removal of half of the seed should be all that was needed if the inhibiting factor were purely mechanical. If this factor were chemical rather than mechanical the inhibiting effect might still occur after the removal of one-half of the seed.

The results of this experiment may be seen in table 1. It will be noted that very little or no growth occurred when either the outer or inner layer of the seed coat remained whole, that better germination took place when approximately half of

TABLE 1. *Effect of seed coat removal on embryo growth.*

		Total embryos in group	None or very little growth	Good growth
A	Whole seeds	11	11	..
B	± outer layer of seed coat removed	12	12	..
C	Whole outer layer removed	12	12	..
D	± ½ outer, ± ½ inner layer removed	11	7	4
E	Outer layer, ± ½ inner layer removed	12	7	2
F	Embryo only	12	2	7

the layers of the seed coat were removed, and that best germination occurred when the embryo was completely separated from all surrounding tissue. The retardation of growth observed when any of the seed tissue was left in contact with the embryo might seem to indicate the presence of some inhibiting factor.

In order to test further the presence of this apparent inhibitor, parts of the two layers of the seed coat were placed in the nutrient very close to the excised embryos. It was thought that the inhibiting substance, if any, might diffuse into the agar and so effect embryo growth. The experimental group grew about as well as the controls. These results may be due to the fact that the concentration of the inhibiting substance was too dilute to influence germination.

INTERSPECIFIC CROSSES INVOLVING D. CERATOCAULA.—*Hybrids obtained.*—Since there are ten herbaceous species of Datura there are eighteen possible combinations, including reciprocal crosses, between *D. ceratocaula* and the other nine species. It was found that abortive embryos were formed in every case when *D. ceratocaula* was used as the male parent. The stage of abortion and the frequency of embryos differed with the female. However, with *D. ceratocaula* as the female parent, abortive embryos were found in only three of the possible nine crosses. The male parents of these successful crosses were *D. discolor, D. inoxia* and *D. metel,* all long-styled plants as is *D. ceratocaula.*

A cross was termed unsuccessful if no embryos were obtained after fifty pollinations. In all cases but one the unsuccessful crosses were those in which the male was a short-styled species. The exception was the cross of *D. ceratocaula* × *D. meteloides* in which the styles of both species are long [±135 mm. in *ceratocaula* and ±190 mm. in *meteloides* (Buchholz, Williams, and Blakeslee, 1935)]. In this cross small seedlike bodies were present which showed no embryos upon dissection. In two of the other unsuccessful crosses, *D. ceratocaula* × *D. stramonium* and *D. ceratocaula* × *D. quercifolia,* the ovules were found occasionally to have enlarged slightly, but not, however, to the extent of those from the *D. ceratocaula* × *D. meteloides* cross. In the other crosses, *D. ceratocaula* × *D. ferox, D. ceratocaula* × *D. pruinosa,* and *D. ceratocaula* × *D. leichhardtii,* the capsules contained only "dust," that is, the remains of ovules such as are found in unpollinated capsules.

The abortive embryos which were found have not all reached maturity. Table 2 shows the results so far. To date, eight of the nine hybrid embryos with *ceratocaula* as the male parent, and one of the three with *ceratocaula* as the female parent have grown to maturity. Thus all nine species hybrids have been obtained. Occasionally an accidental self pollination or the development of a haploid embryo occurred, but these could be distinguished readily in the case of the *ceratocaula* crosses at the time of dissection.

Embryo frequency.—The frequency of embryos in the different crosses varied greatly as might be expected. The two crosses regularly producing the largest proportion of embryos were *discolor* × *ceratocaula* and *ferox* × *ceratocaula.* Of the other crosses with *ceratocaula* as the male parent, those with *inoxia, pruinosa, quercifolia, leichhardtii II, stramonium,* and *meteloides* produced embryos in moderate numbers, while embryos were found very rarely in the crosses with *leichhardtii I* and *metel.* Not enough dissections were made in the successful crosses involving *ceratocaula* as the female parent to determine the frequency of embryos in these crosses.

TABLE 2. *Results of crosses between D. ceratocaula and the other nine Datura species. H—Dissected embryo grown to a mature hybrid. E—Dissected embryo not yet grown to a mature hybrid. (—)—No embryos obtained.*

	Stramonium	*Quercifolia*	*Ferox*	*Leichhardtii*	*Pruinosa*	*Discolor*	*Inoxia*	*Metel*	*Meteloides*
Ceratocaula ♂	H	H	H	H	H	H	H	E	H
Ceratocaula ♀	(—)	(—)	(—)	(—)	(—)	E	E	H	(—)

The cross *leichhardtii* × *ceratocaula* was the only one in which all the seeds were dissected in a sufficient number of capsules to give a reliable percentage of capsules containing embryos. Here there was an evident difference between the two races of *leichhardtii*. The results of the crosses are found in table 3. With *leichhardtii I* there was an average of 1.9 embryos per 100 seeds, and with *leichhardtii II* the average was 16.1 embryos per 100 seeds. That is, embryos from the last cross were almost nine times as frequent as those from the first. Thirty-eight per cent of the capsules of *leichhardtii I* were found to contain one or more embryos, while 85 per cent of the capsules of *leichhardtii II* had embryos. These experiments with the two *leichhardtii* races were carried on at the same time and under the same conditions. Blakeslee and Satina (1944) found that differences exist in the crossability of the two *leichhardtii* races in the cross *leichhardtii* × *discolor*. Here they found that viable seeds were produced when the female was *leichhardtii I* but only arrested embryos were formed when the female was *leichhardtii II*. Thus in crosses with *discolor, leichhardtii I* gave the best results as the female parent, and in crosses with *ceratocaula, leichhardtii II* gave the best results. In other crosses of *ceratocaula* with several different types of one species a thorough study has not been made to determine whether or not similar racial differences in embryo production exist. Apparently such differences do not exist in the cases of *stramonium, ferox,* and *inoxia*.

In *stramonium* over ninety different chromosomal types have been isolated, a few from nature but most produced by radiation (Bergner, Satina and Blakeslee, 1933). During a period of several years more than five hundred embryos of the cross *stramonium* × *ceratocaula* involving a total of thirty-six types have been cultured. While results show that certain types yielded a much higher percentage of capsules containing embryos than others this cannot be taken at face value. Table 4 gives data on the percentage of capsules with embryos together with the pollination dates. It will be noted that four days stand out from the other nine as having been good days for pollination. The fact that certain days are better than others for pollination has been substantiated by other crosses involving *stramonium*. This does not hold for other *ceratocaula* crosses. An examination of the dates shows that the most successful crosses happened to be made on these four particularly good days. From these data it would seem that it was the day rather than the type that was influencing embryo production. Whether or not the chromosomal type in *stramonium* has any influence would have to be determined by making pollinations with the different types on the same days. The cause of the daily variations is not known. Since a number of plants are affected at the same time it would seem that external causes such as changes in sunlight, temperature, and humidity might be the deciding factors. It should be pointed out that the *stramonium* crosses differ from those with *leichhardtii* in that many of the chromosomal types of *stramonium* were produced artificially from one original line while the two *leichhardtii* races came from nature and had different gene complexes.

TABLE 3. *Results of crosses between D. ceratocaula and the two types of D. leichhardtii.*

Female parent	Capsules	Seeds dissected	Embryos	% seeds with embryos	% capsules with embryos
D. leichhardtii I	60	1732	33	1.9	38.3
D. leichhardtii II	27	596	96	16.1	85.2

TABLE 4. *Pollination dates and percentage of capsules with embryos from the cross D. stramonium* × *D. ceratocaula.*

Pollination date	Capsules with embryos	Total capsules	% capsules with embryos
4/ 3/44	5	18	28
4/ 7/44	2	10	20
4/10/44	14	15	**93**
5/ 8/44	4	26	15
5/12/44	11	38	29
10/16/44	4	21	19
10/19/44	3	22	14
10/27/44	4	10	40
10/29/44	23	31	**74**
11/10/44	2	10	20
11/14/44	13	20	**65**
11/16/44	5	10	50
11/27/44	7	11	**64**

Embryo forms.—The normal selfed embryos of the various species of Datura attain full size from 3 to 5 weeks following pollination (fig. 10–11) after which they go through a process of maturation. The full sized embryo has well developed hypocotyl and cotyledons. As an example of the difference in speed of embryo growth in various species, the *ceratocaula* embryo may be compared with that of *stramonium*. The *stramonium* embryo (Satina, Blakeslee, and Avery, 1938) reaches the "heart" stage, in which the cotyledons are just beginning to differentiate, about 13 days after pollination, and by 20 days after pollination the embryo is nearing its full size. The *ceratocaula* embryo, on the other hand, does not reach the "heart" stage until 18 to 20 days after pollination while another 6 to 7 days elapse before it attains full size. This rate of growth is that of the most mature embryos, as some variability occurs among seeds in the same capsule. There are also variations among capsules probably due to environmental conditions, but this is the general pattern.

The arrested hybrid embryos when removed from the seed rarely showed normal development (fig. 1–9). Their form varied from a completely undifferentiated mass to a differentiated but often misshapen embryo. No crosses with *ceratocaula* as the female parent yielded differentiated embryos, but

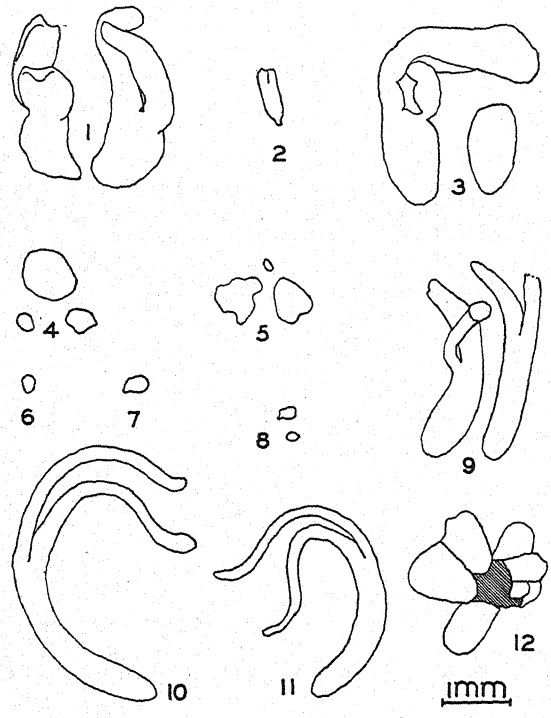


Fig. 1–12.—Fig. 1–11. Camera lucida drawings of embryos to same scale at time of dissection.—Fig. 1–9. Hybrid embryos between *D. ceratocaula* ♂ and the following species.—Fig. 1. *D. stramonium.* 24 days after pollination.—Fig. 2. *D. quercifolia.* 21 days.—Fig. 3. *D. ferox.* 24–26 days.—Fig. 4. *D. leichhardtii.* 24–26 days.—Fig. 5. *D. inoxia.* 26 days.—Fig. 6. *D. meteloides.* 28 days.—Fig. 7. *D. metel.* 38 days.—Fig. 8. *D. discolor.* 25 days.—Fig. 9. *D. pruinosa.* 26 days.—Fig. 10. Normal embryo from selfed *D. discolor.*—Fig. 11. Same from *D. pruinosa.*—Fig. 12. Hybrid embryo from cross *D. leichhardtii* × *D. ceratocaula* showing growth of many buds from a central mass after ten days in culture.

such were found in four crosses in which *ceratocaula* was the male parent, namely, with *stramonium, ferox, quercifolia,* and *pruinosa.* Hybrid embryos of the first two of these crosses were usually found flattened with hypocotyl and cotyledons growing abnormally. Embryos of the last two crosses were quite normal in appearance for their stage of growth but were materially slowed in development. Embryos of the other crosses were generally undifferentiated bodies (*leichhardtii* × *ceratocaula, discolor* × *ceratocaula, inoxia* × *ceratocaula, metel* × *ceratocaula, meteloides* × *ceratocaula, ceratocaula* × *inoxia, ceratocaula* × *discolor,* and *ceratocaula* × *metel*). In two of these crosses which produced undifferentiated embryos, *leichhardtii* × *ceratocaula* and *inoxia* × *ceratocaula,* two or more embryos were often found in one seed. Sometimes these were entirely separate, as frequently occurred in the cross *leichhardtii* × *ceratocaula,* and other times several "lumps" of tissue seemed fused into one embryo, as was frequent in the cross *inoxia* × *ceratocaula.*

In most of the crosses at the time of dissection, 3 to 4 weeks after pollination, all traces of endosperm had disappeared, but the embryo had not yet been injured by the apparent lack of moisture. The endosperm was not always absent. In the cross *inoxia* × *ceratocaula* the embryo was often surrounded by a watery jelly-like substance, which was decidedly different from the endosperm of a selfed seed at a comparable age.

Embryo growth.—Growth of the embryos in nutrient varied greatly among the different species hybrids and was not necessarily connected with the degree of differentiation of the embryo. The best growth among the nine crosses in which *ceratocaula* was the male parent was shown by the embryos from the two crosses *inoxia* × *ceratocaula* and *discolor* × *ceratocaula.* Embryos from both of these crosses were undifferentiated bodies at the time of dissection. Embryos from the cross *metel* × *ceratocaula* (also undifferentiated bodies) have so far shown no signs of growth in nutrient, while the other six crosses including the four giving differentiated embryos fall between these two extremes. These growth differences are probably due to different growth requirements which as yet have not been determined. The number of embryos dissected from crosses in which *ceratocaula* was the female parent was insufficient to show true growth variations.

Multiple growth of an embryo in nutrient occurred quite frequently in certain crosses (*leichhardtii* × *ceratocaula, inoxia* × *ceratocaula, discolor* × *ceratocaula*) while in other crosses it occurred rarely (*stramonium* × *ceratocaula*) or not at all (*ferox* × *ceratocaula, quercifolia* × *ceratocaula,* and *pruinosa* × *ceratocaula*). In the cases which showed this peculiarity, buds, produced from a central undifferentiated mass (fig. 12), eventually broke off and became individual seedlings. It was not unusual to obtain half a dozen or more separate cultures by this process. In one instance an extreme form of multiple growth was noted. From a seed of the cross *ceratocaula* × *metel* an undifferentiated embryo less than one third of a millimeter was removed. From this body over a period of several months one hundred and six buds were isolated. As soon as one bud grew big enough, it broke off thus leaving room for others to grow. Many of this number produced roots and shoots and were grown to mature plants. Undoubtedly many more could have been so grown had they been desired.

Mature hybrids.—The nine mature hybrids secured had a very high percentage of aborted pollen grains and therefore were practically sterile. Almost all of the capsules fell off when the flowers were self-pollinated. The very few that persisted

were very small and contained at most only two or three seeds.

The fact that these nine hybrids from such wide crosses all had one parent in common offered an excellent opportunity for study of the characters in the F_1 plants. As already pointed out *ceratocaula I* was the race used in these experiments. The parents with which *ceratocaula* was crossed were individuals in highly inbred races: *stramonium, ferox I, quercifolia I, leichhardtii II, inoxia II, metel III, pruinosa I,* and *discolor I*. The description of the *stramonium* hybrids is not confined to any particular race since there were no evident morphological differences in the races involved. The hybrid with *meteloides* is not included in the following descriptions.

The characters as expressed in the hybrids may be grouped into four main classes and these in some cases subdivided as shown below. Group *A* includes *ceratocaula* characters which are dominant in the hybrids; group *B* hybrid characters which are intermediate between those of the two parents; group *C ceratocaula* characters which are recessive in the hybrids; and group *D* hybrid characters which are not evident in either parent.

The differences existing between the various species of Datura and between these and the hybrids with *ceratocaula* may be summed up as either color or morphological differences. Many of the latter differences have to do with the presence or absence of hairs on various parts of the plant. Each of the groups listed above includes some of both types of characters.

The five *ceratocaula* characters dominant in all hybrids were sympodial branching (above the basal whorl), hollow stem, absence of pedicel hairs, purple vein in anther, and purple pigmentation on the inner walls of capsules. It may be mentioned that this last character showed only very faintly in hybrids between *ceratocaula I* and *ceratocaula II* which, like the other Datura species, has no purple pigment on the inner capsule walls.

Those characters of *ceratocaula* which were dominant in some but not all of the hybrids were whorled branching at base, lack of indentation of petals, and purple color of filaments at the point of attachment to the corolla. The expression of whorled branching was questionable in some of the hybrids due possibly to environmental conditions including vigor of the individual plant. While it undoubtedly did occur in certain cases, it was sometimes difficult to determine in others, when there were only one or two plants, whether branching was dichotomous, or whorled and sympodial. *Ceratocaula* itself sometimes branches dichotomously, especially if it is not growing under favorable conditions. In the other species which are dichotomous there is often a third undeveloped shoot at the point of branching. This was sometimes the case in the hybrids in which the method of branching was difficult to classify. The flowers of all the herbaceous Daturas are sympetalous, but in some species there are indentations at the edges of the corolla which serve to emphasize the five-parted flower. These are present in the corollas of the four species *stramonium, ferox, quercifolia* and *metel*. They showed very slightly in the corollas of the hybrids with *stramonium* and *ferox,* but were not evident in any of the other hybrids. The purple color at the point of attachment of filaments and corolla is present in the one species, *ceratocaula*. It showed up constantly in the hybrids with *stramonium* and with *inoxia* and irregularly in those with *ferox* and with *quercifolia*. There was no evidence of the purple coloring at attachment points in the other hybrids.

There were a few characters of *ceratocaula* which always appeared in the hybrids but which were lacking in some of the other species. Capsules, for example, are erect in the three species, *stramonium, quercifolia,* and *ferox,* semi-erect in *metel,* and nodding in all the others. All hybrids with *ceratocaula,* however, had nodding capsules. Hairs are present on the portion of the filament attached to the corolla in all species except *inoxia*. This was true of all hybrids including *inoxia* × *ceratocaula*.

Some hybrid characters were intermediate between those of the two parents. This was true of the number of hairs on young buds in all hybrids. In all species, with the exception of *ceratocaula,* hairs are very numerous on young buds. In *ceratocaula* they are either absent altogether or present only near the tip. In the buds of the hybrids, hairs were more numerous than in *ceratocaula* but not as numerous as in the other parents. The number of hairs on the ventral leaf surface was a character which was generally intermediate in the hybrids. The ventral surface of the *ceratocaula* leaf is white with appressed hairs. In no other species are they nearly as numerous. All the hybrids seemed to be more or less intermediate in this respect.

A character which was intermediate in some of the hybrids was the shape of the leaf. In order to get an accurate index of this, the ratio of width to length of leaf was plotted for a number of leaves of each species and each species hybrid. The *ceratocaula* leaf is quite narrow in comparison with the leaves of all other species. Two hybrids, *discolor* × *ceratocaula* and *quercifolia* × *ceratocaula,* produced leaves intermediate between their parents. The two female parents have on the average the widest leaves of all the species. All the other hybrids had leaves which were nearer the shape of the wide-leafed parent. Other characters which were sometimes intermediate in the hybrids were corolla length and filament length. This was true only for some hybrids involving the small flowered species. The large flowered species produced hybrids with corollas and filaments longer than either parent. The hybrid with *stramonium,* which has a corolla of intermediate length, had a corolla nearly as long as that of *ceratocaula*. With the larger flowered species, therefore, there seems to be some evidence of hybrid vigor so far as flower size is concerned.

A number of *ceratocaula* characters were reces-

sive in all hybrids. These were the deep yellow stigma color, smooth ovary and capsule, enlarged peduncle, absence of a thickened persistent calyx base, and absence of hairs on the capsule surface. The stigmas of all other species except *inoxia* are green (with purple papillae scattered over the surface in those of *stramonium* and *metel*). The stigma of *inoxia* is cream colored. Those of the hybrids (except *inoxia* × *ceratocaula*) were green (with purple papillae when present in the parent). They showed at most a slight yellow tinge around the edges. The stigma of the hybrid, *inoxia* × *ceratocaula*, was a pale yellow. All species except *ceratocaula* and all hybrids had ovaries with soft spines and capsules with spines of various sizes. The spines of the hybrids were in no case as large as those of the parent. The peduncle of *ceratocaula* is enlarged and covers almost the whole base of the capsule while those of the other species are not enlarged. No enlargement occurred in the hybrids. The thickened base of the calyx persists in all species except *ceratocaula*. It may be recurved or at right angles to the capsule, or it may curve inward. All hybrids had persistent calyx bases which, with the exception of *stramonium* × *ceratocaula*, were incurved and often partially enclosed the capsule. The calyx base of this hybrid was at right angles or recurved, more or less like that of the female parent. Hairs are present in varying number on the capsules of all species except *ceratocaula*. Hairs were also present on all hybrid capsules, but were usually less numerous than those of the other parent species.

The narrow shape of leaf of *ceratocaula* was usually recessive in the hybrids (intermediate in two cases mentioned above).

There were three characters which never appeared in *ceratocaula* but which did appear in some of the other species and in the hybrids with these species. These involved anther color, ovary color, and prominence of calyx veins. The anthers are white in all species except two. In purple races of *stramonium* they are dark purple and in *quercifolia* they are blue. The two hybrids with these species produced anthers which were the same color but a lighter shade than the female parent. In all species except *discolor* and *inoxia* the ovaries are green. In these two species and their hybrids they are straw colored. The veins of the calyx stand out prominently in the five species: *stramonium, ferox, quercifolia, discolor,* and *metel,* and in the hybrids with these species. The other four species, including *ceratocaula,* and the hybrids have inconspicuous calyx veins.

The last group of characters are those which were present in some of the hybrids but which did not show in either parent. The free portion of the filament is white or green in all species and in all hybrids with the exception of *ceratocaula* × *metel*. In this hybrid it was purple. Another character having to do with color is concerned with the filament hairs which are present on the part of the filament attached to the corolla in all species except *inoxia,* and which when present are colorless. There were three species hybrids (*inoxia* × *ceratocaula, discolor* × *ceratocaula,* and *ceratocaula* × *metel*) in which the hairs were irregularly purple. In the other hybrids they were colorless like those of the parents. One character in this group which seemed to hold for all hybrids (with the possible exception of *ceratocaula* × *metel* for which evidence is lacking) was the peculiarly "waxy" appearance on the surface of all hybrid capsules irrespective of presence of hairs. This character does not appear in the capsules of any of the parental species.

The above characters may be summarized as follows. *A. Ceratocaula* characters dominant in all hybrids: sympodial branching, hollow stem, absence of pedicel hairs, purple vein in anther, and purple pigment on inner walls of capsules. *Ceratocaula* characters dominant in some hybrids: whorled branching at base of stem, lack of indentation of petals, and purple pigmentation of filament at point of attachment to corolla. *Ceratocaula* characters, when different in the parents, dominant in the hybrids: nodding capsules and presence of hairs on portion of filament attached to corolla. *B.* Hybrid characters always intermediate between those of the two parents: number of hairs on small buds and number of hairs on ventral leaf surface. Hybrid characters sometimes intermediate between those of the two parents: narrowness of leaf and length of corolla and filament. *C. Ceratocaula* characters recessive in all hybrids: deep yellow stigma color, absence of spines on ovary and capsule, enlarged peduncle, absence of thickened persistent base of calyx, and absence of hairs on capsule. A *ceratocaula* character recessive in some hybrids: narrowness of leaf. *Ceratocaula* characters, when different in the parents, recessive in the hybrids: white anthers, green ovary, prominent calyx veins. *D.* Characters in hybrids not evident in either parent: purple pigmentation of free portion of filaments, purple pigmentation of filament hairs, and "waxy" surface of capsules.

Discussion.—It has been shown in this paper that certain interspecific hybrids of Datura hitherto thought impossible have become obtainable through the technique of embryo dissection and culture. Of the eighteen combinations between *ceratocaula* and the other nine species, twelve have given dissectable embryos.

This does not mean that no fertilization took place in the other six combinations, but only that if fertilization did occur, the embryos aborted before they reached a dissectable size, around 0.1 mm. All but one of the six unsuccessful cases involved the growth of pollen tubes of a short-styled plant through the style of a long-styled plant. The one exception was the cross *ceratocaula* × *meteloides*. In this case the male parent averaged a longer style than the female. It may be significant that this cross produced more and larger seed-like bodies than any of the other five. Two other crosses also pro-

duced a few seed-like bodies (*ceratocaula* × *quercifolia* and *ceratocaula* × *stramonium*). This cannot be taken as an indication of fertilization until sections have been made and studied. In three crosses (*ceratocaula* × *ferox, ceratocaula* × *pruinosa,* and *ceratocaula* × *leichhardtii*) no enlarged ovules were noted. This is probably a fairly good indication that no fertilization took place, but here again sections would have to be made. It is possible that the pollen tube growth may stimulate the development of ovules without fertilization actually taking place. It was found by van Overbeek, Conklin and Blakeslee (1941) that ovule development could be induced in unpollinated capsules of *stramonium* by the injection into the ovary of naphthaleneacetic acid or indolebutyric acid. There may well be some chemical stimulus from the growth of pollen tubes which acts in a similar way in inducing ovule development.

If the results of the unsuccessful crosses with *ceratocaula* are compared with the results of Buchholz, Williams, and Blakeslee (1935) on interspecific pollen tube growth, correlation can be found for some crosses, not for others. These authors have studied the growth and percentage of bursting of the pollen tubes in the Datura species. Diagrams show the pollen-tube distribution in the style of the female parent for all combinations at a certain interval after pollination. Germination of pollen on the stigma of *ceratocaula* took place in each of the unsuccessful crosses. However, the pollen tubes of only two species grew to any extent after germination. Those of *meteloides* (the only long-styled species of this group) grew best and showed the least amount of bursting. The *pruinosa* pollen tubes grew to some extent but many tubes burst. The pollen tubes from the other four species, *stramonium, quercifolia, ferox,* and *leichhardtii,* grew extremely poorly, and a large percentage burst. This does not mean that any of the pollen tubes of *meteloides* or *pruinosa* reached the ovary but only that at a certain time after pollination they were found to be growing in the style.

The fairly good pollen-tube growth in the cross *ceratocaula* × *meteloides* may be related to the presence of the seed-like bodies which were found in the capsule. Likewise, the extremely poor growth of *leichhardtii* pollen tubes in the style of *ceratocaula* may be related to the absence of enlarged ovules in this particular cross. However, the other results do not agree. Seed-like bodies were found occasionally in the capsules of *ceratocaula* × *stramonium* and *ceratocaula* × *quercifolia,* but pollen-tube growth in both crosses was very poor. No seed-like bodies were found in the capsules of *ceratocaula* × *pruinosa* although pollen-tube growth in this cross is better than in the two above.

If short pollen-tube growth were the determining factor in non-fertilization it would perhaps be possible to remove the barrier by shortening the style. Successful style splicing was done by Buchholz, Doak and Blakeslee (1932). Buchholz also obtained pollen germination and pollen-tube growth by splitting a decapitated style and pollinating the cut surface. This latter method was tried to a limited extent by the writer but with no positive results. However, more work would have to be done to give this technique a fair trial.

SUMMARY

The very poor seed germination of *D. ceratocaula* has been increased markedly by culturing the embryos in an agar nutrient of sucrose and inorganic salts.

Arrested embryos were found to occur in each of the nine possible interspecific crosses with *ceratocaula* as the male parent and in only three of the nine possible crosses with *ceratocaula* as the female parent.

Through use of the embryo dissection and culture technique, eight of the nine possible hybrids involving *ceratocaula* as the male parent and one involving *ceratocaula* as the female parent have been grown to maturity. This latter hybrid is the one lacking in the first group. Therefore all nine hybrid combinations have been obtained.

Racial differences affect crossability as shown by the fact that one race of *D. leichhardtii* gives over eight times as many arrested embryos as another race when pollinated by *ceratocaula.*

The arrested embryos at the time of dissection varied with the cross from small undifferentiated bodies less than one third of a millimeter to large differentiated embryos four or five millimeters in length. They also varied greatly in frequency and in growth patterns.

Some *ceratocaula* characters are dominant in hybrids with other species, some intermediate, some recessive, while in some cases the hybrid has characters not evident in either parent.

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EFFECT OF HYDROXYPROLINE ON TRICHOPHYTON MENTAGROPHYTES AND OTHER FUNGI [1]

William J. Robbins and Ilda McVeigh

IN A PREVIOUS paper from this laboratory (Robbins and Ma, 1945) small amounts of hydroxyproline were reported to inhibit the growth of *Trichophyton mentagrophytes* (*T. gypseum*). Marked inhibition was observed with one part of hydroxyproline in 8000 parts of medium and complete inhibition with one part in 800. Schopfer and Blumer (1943) found that *T. album* also was inhibited by hydroxyproline. Growth was reduced about 70 per cent by the addition of one part of hydroxyproline in 20,000 parts of medium and completely inhibited by 1 in 10,000. Since this amino acid is a common constituent of proteins and relatively small amounts exerted a deleterious effect on these fungi, it seemed desirable to explore further the relation of hydroxyproline to the growth of fungi.

MATERIALS AND METHODS.—Most of the work reported here was carried on with a strain of *T. mentagrophytes* isolated from a human subject in 1942. It has been maintained in its original freely sporulating condition by transferring to fresh media at short intervals, usually 7 to 10 days. The original form is designated, T-689 (N). As is common in this group pleomorphic forms arise as the cultures age. Two such forms, T-689 (A) and T-689 (B), were used in this study.[2] A third, T-689 (BB), derived from T-689 (B) was included in the investigation (fig. 1). Some observations were made also on *Epidermophyton flocculosum* #45–92, *Trichophyton purpureum* #297, *T. gypseum* (granular type) and *Microsporum canis* kindly supplied by Dr. Rhoda W. Benham. In addition, we used, in some experiments nineteen fungi, listed later in this paper, selected at random from the great groups.

The fungi were grown on a basal medium supplemented with various sources of nitrogen and in some experiments with a vitamin mixture. Stock cultures were maintained on a thiamine-peptone agar.[3] For some experiments the fungi were grown on agar slants in test tubes each containing 8 ml. of medium solidified with specially purified agar. Colony diameters were measured and general observations made after 4 and 7 days and at weekly intervals thereafter for 2 or 3 weeks. For other experiments the fungi were cultivated in 125 ml. Erlenmeyer flasks containing 25 ml. of liquid media. Dry weights were determined after autoclaving the cultures by filtering into Gooch crucibles, washing with distilled water and drying at 100°C. In some instances, *T. mentagrophytes* was grown in Erlenmeyer flasks containing 10 ml. of agar media. Water lost from these cultures during incubation was replaced at intervals of about 5 days by the addition of sterile distilled water. When dry weights were determined for the agar cultures distilled water was added to each flask. After autoclaving the mycelium was washed free of agar by hot water, dried in Gooch crucibles and weighed.

Test tube cultures of *T. mentagrophytes* were inoculated with a bit of mycelium and spores about as large as a pin head. Somewhat more inoculum was used for those cultures where dry weights were determined. All cultures were run in triplicate. The results of all experiments reported in this paper were supported by other comparable tests.

The glassware was Pyrex, cleaned with chromic acid-sulfuric acid mixture, and thoroughly rinsed with tap and distilled water. The chemicals were of C. P. grade.

Hydroxyproline and growth of T. mentagrophytes.—The effect of hydroxyproline on the growth of T-689 (N) was determined in test tubes containing 8 ml. of the basal agar medium with 2 g. of asparagine per l.; in 125 ml. Erlenmeyer flasks containing 25 ml. of the basal liquid medium with asparagine and in 125 ml. Erlenmeyer flasks containing 10 ml. of the basal agar medium with asparagine.

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This investigation was supported in part by a grant from the Albert and Mary Lasker Foundation, Inc. This assistance is gratefully acknowledged by the authors.

[2] T-689 (A) and T-689 (B) were derived from forms designated (A) and (B) in the earlier paper (Robbins and Ma, 1945) but are not identical with them. Failure to transfer the original mutants at short enough intervals resulted in mutation of the original (A) and (B) forms.

[3] The basal solution was the same as that used earlier (Robbins and Ma, 1945) except that asparagine was omitted. When asparagine was used it was added at the rate of 2 g. per l. The hydrion concentration was adjusted to pH 5.4–5.6 by the addition of NaOH. The thiamine-peptone agar, vitamin mixture, casein hydrolysate and preparation of purified agar are described in the earlier paper.

In test tubes after 2 weeks growth, 0.1 mg. (1–80,000) of hydroxyproline (Eastman no. 3594) had little effect; 0.2 mg. (1–40,000) caused some inhibition and 0.4 mg. (1–20,000) complete or nearly complete inhibition (fig. 2). After 3 weeks in liquid cultures growth as measured by dry weights was decreased little or none by 1–40,000 or less but was markedly reduced by 1–20,000 or more. After 16 days in agar cultures dry weights were little affected by 1–40,000 or less and markedly reduced by 1–20,000 or more (table 1).

T-689 (A) and T-689 (B) were grown in test tubes on the basal agar medium and in liquid cultures both containing asparagine. Both pleomorphic forms were more resistant to hydroxyproline than the normal form. In test tubes (fig. 2) and in liquid culture (table 1) the growth of T-689 (A) decreased as the hydroxyproline was increased from 0.4 mg. (1–20,000) to 2 mg. (1–4,000) per tube and was completely inhibited by 4 mg. (1–2,000). In test tubes growth of T-689 (B) was not affected by 0.4 mg. (1–20,000) or less per tube, was reduced by 1.0 mg. (1–8,000) and completely inhibited by 2.0 mg. (1–4,000). In liquid cultures 0.625 mg. (1–40,000) or less had no effect, 1.25 mg. (1–20,000) reduced the growth slightly and 3.125 mg. (1–8,000) produced almost complete inhibition (table 1).

The hydroxyproline was fungistatic rather than fungicidal. This was demonstrated by transferring the inoculum from a tube containing 5 mg. (1–1,600) of hydroxyproline after 10 days incubation to a tube of thiamine-peptone agar. The inoculum which had shown no growth in the tube containing hydroxyproline for a period of 10 days grew on the thiamine-peptone medium.

No difference was noted between the effect of hydroxyproline autoclaved with the medium, autoclaved separately and added to the balance of the medium or filtered sterile and added to the medium.

Hydroxyproline and other dermatophytes.—The effect of hydroxyproline on four other dermatophytes was determined in test tube cultures using the basal agar medium with asparagine to which 0.0, 0.2, 0.4, 1.0, 2.0, or 5.0 mg. of hydroxyproline were added per tube. The cultures were observed over a period of 18 days. All four dermatophytes showed evidences of injury from the addition of the amino acid (fig. 3) but differed in their responses as follows:

TABLE 1. *Average dry weight of triplicate cultures of three forms of T. mentagrophytes on a basal agar (10 ml.) or in liquid medium (25 ml.) containing asparagine and various amounts of hydroxyproline.*

Hydroxyproline added to basal medium	Average dry weight per culture, mg. T-689 (N) 3 weeks in liquid	T-689 (N) 16 days on agar	T-689 (A) 2 weeks in liquid	T-689 (B) 2 weeks in liquid
None	2.2	9.4	14.0	90.5
1 to 60,000	2.2	10.7	13.2	87.5
1 to 50,000	1.7	9.9	10.2	75.3
1 to 40,000	1.6	8.8	14.7	96.7
1 to 20,000	0.6	2.6	8.9	70.6
1 to 8,000	0.3	1.0	4.8	0.6
1 to 6,000	0.1	0.4	2.9	0.7
1 to 4,000	0.3	0.4	1.4	2.3
1 to 2,000	0.3	0.4	0.7	0.6
1 to 1,500	0.1	0.4	0.8	0.4

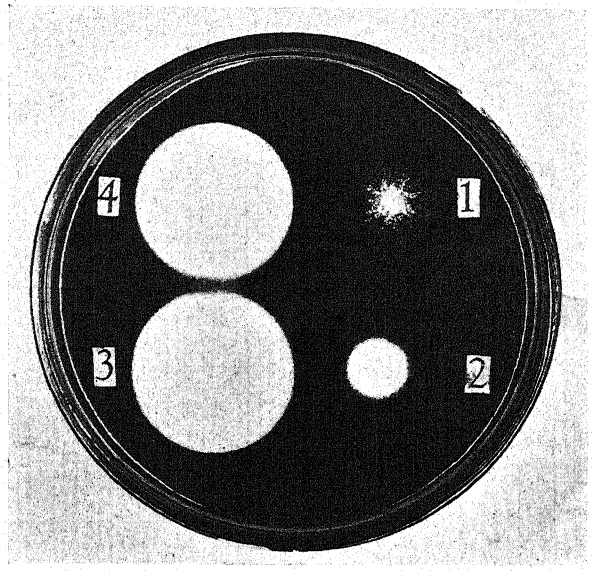


Fig. 1. Normal and pleomorphic forms of *T. metagrophytes* grown 14 days on a basal agar medium containing asparagine. 1, T-689 (N); 2, T-689 (A); 3, T-689 (B); 4, T-689 (BB).

Trichophyton gypseum (granular form) was somewhat more resistant than T-689 (N). Good growth was obtained in tubes containing 0.4 mg. (1–20,000) and complete inhibition with 1.0 mg. (1–8,000).

T. purpureum was stimulated by 0.2 mg. and 0.4 mg.; 1.0 mg. had little effect; considerable inhibition was observed in the tubes containing 2.0 mg. (1–4,000) and complete inhibition in the tubes with 5.0 mg. (1–1,600).

Microsporum canis. Growth was decreased by 1.0 mg. (1–8,000) but not completely inhibited by 5.0 mg. (1–1,600). In fact, the growth in the latter tubes was about one-half that obtained on the basal medium.

Epidermophyton flocculosum was the most sensitive to the injurious effects of hydroxyproline. Nearly complete inhibition was obtained with 0.2 mg. (1–40,000), the smallest amount used.

Various samples of hydroxyproline.—All samples of hydroxyproline available to us inhibited growth but not all were equally effective on T-689 (N). Some induced complete inhibition at 1–20,000 in the asparagine agar medium; others permitted some growth even at concentrations of 1–1,600.

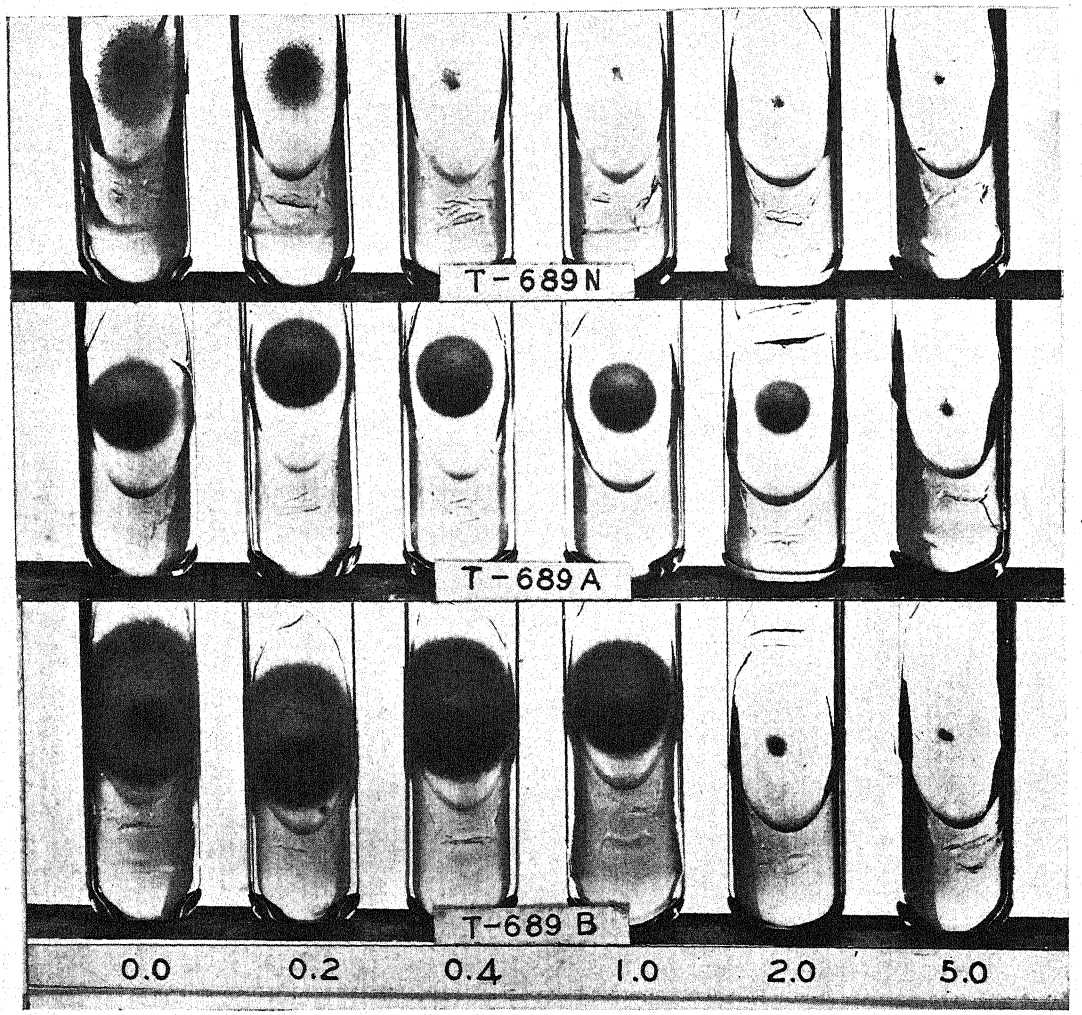


Fig. 2. Normal and pleomorphic forms of *T. mentagrophytes* grown 13 days on the basal agar medium containing asparagine. Hydroxyproline was added at 0.0, 0.2, 0.4, 1.0, 2.0 or 5.0 mg. per tube.

The results obtained with 9 different samples of hydroxyproline at 5 mg. per tube in the basal agar medium with asparagine are shown in table 2. Two of the samples, Merck no. 42191 and Eastman no. 3594, caused complete inhibition.

TABLE 2. *Colony diameter of T-689 (N) on basal agar medium containing asparagine and 5 mg. per tube of various samples of hydroxyproline. Age 14 days.*

Source of hydroxyproline	Colony diameter
	mm.
Merck #40703	13
Merck #42845	21
Merck #42191	5
Merck #3R4132	18
Eastman #3594	6
Dr. Chester Stock	23
Hoffman-La Roche #306057	20
Paragon I	15
Paragon II	21
No hydroxyproline	28

A pleomorphic strain resistant to hydroxyproline.—In a tube containing 1–1,600 hydroxyproline and inoculated with T-689 (B) evidence of renewed growth was observed after 19 days. The isolation made from this proved to be quite resistant to hydroxyproline; good growth was obtained on the basal agar medium with asparagine to which 50 mg. (1–160) of hydroxyproline had been added. This isolation was designated T-689 (BB).

Hydroxyproline and other fungi.—Other fungi were grown on an agar medium in test tubes and on the same medium containing 1–80,000, 1–8,000 or 1–1,600 hydroxyproline. *Agaricus campestris, Aspergillus niger, Ceratostomella ulmi, Neurospora sitophila* 56–2, *Penicillium notatum, Phycomyces blakesleeanus, Rhodotorula aurantiaca, R. sp.* and *Stereum murrayi* were grown at 20°C. on the basal agar medium with asparagine and the vitamin mixture. *Polyporous alboluteus* (72163), *P. berkeleyi*

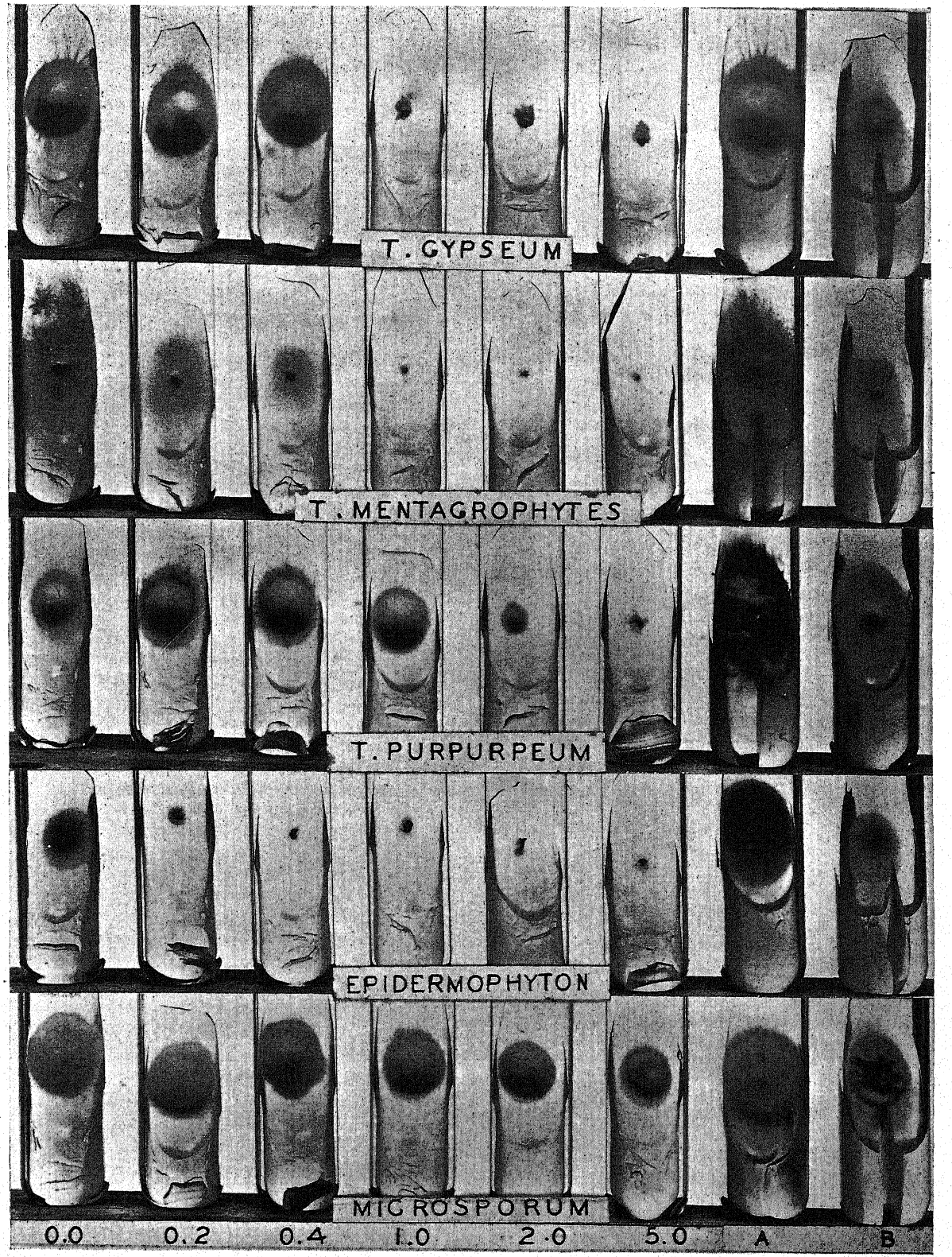


Fig. 3. Various dermatophytes grown 18 days on the basal agar medium containing asparagine. Hydroxyproline was added at 0.0, 0.2, 0.4, 1.0, 2.0 or 5.0 mg. per tube; 1.0 mg. of casein hydrolysate to tubes, A, and 1.0 mg. of casein hydrolysate plus 10.0 mg. hydroxyproline to tubes, B.

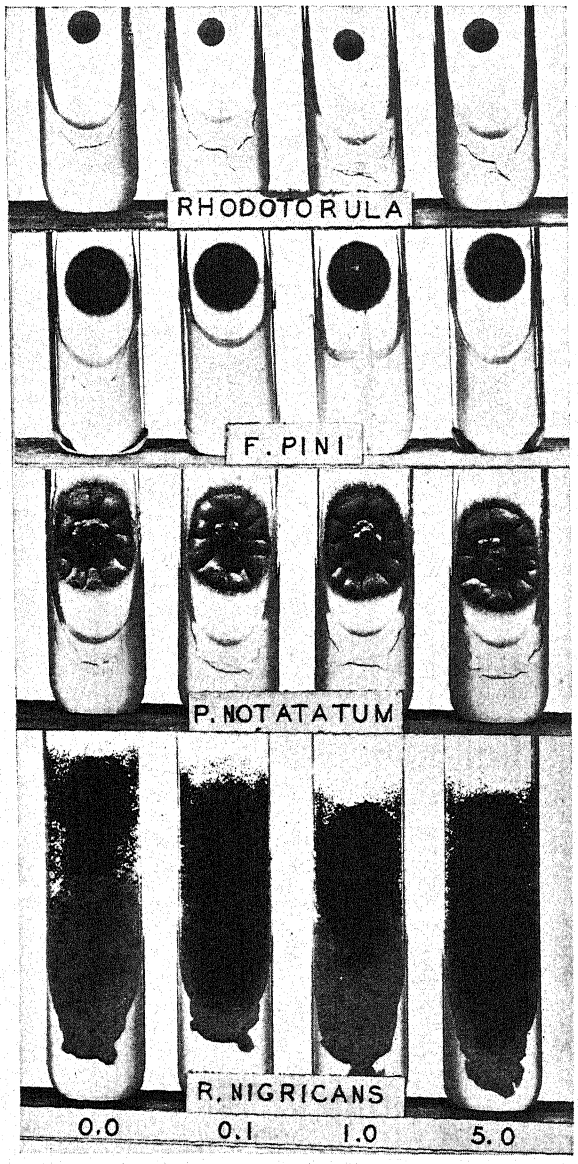


Fig. 4. Effect of hydroxyproline on *Rhodotorula* sp., *Fomes pini*, *Penicillium notatum* and *Rhizopus nigricans* grown on basal agar medium plus asparagine, vitamin mixture and, for *F. pini*, 1.0 mg. of casein hydrolysate per tube. Hydroxyproline added at 0.0, 0.1, 1.0 or 5.0 mg. per tube. Compare with figure 2 and figure 3. Age 6 days except *F. pini* which was 26 days old.

(53379-S), *P. circinatus* (72076-S) *P. croceus* (47479-S), *P. obtusus* (71791-S), *P. schweinitzii* (94095-R), *P. squamosus* (39296), *Fomes pini* (52061) and *F. pomaceus* (2283) were grown at 25°C. on the basal agar medium supplemented with the vitamin mixture and 1 mg. of casein hydrolysate per tube. None of these organisms showed any response to hydroxyproline except *Polyporous squamosus* (fig. 4, 5). The growth of this fungus was not affected by 0.1 or 1.0 mg. of hydroxyproline but was markedly improved by 5.0 mg. (1–1,600).

Hydroxyproline and ammonium nitrate.—In the earlier work of this laboratory (Robbins and Ma, 1945) T-689 (N) was found to grow poorly or not at all with ammonium nitrate as the source of nitrogen, while the pleomorphic forms were able to use the inorganic source of nitrogen. We have found further that the nitrate ion is unavailable to the pleomorphic forms; no growth was obtained in media with $NaNO_3$ as the sole source of nitrogen but the growth with $(NH_4)_2SO_4$ was about equivalent to that obtained with NH_4NO_3.

It seemed desirable to determine whether hydroxyproline would be injurious to *Trichophyton mentagrophytes* if inorganic nitrogen was supplied instead of an organic source, for example, asparagine.

T-689 (A), T-689 (B) and T-689 (BB) were grown in test tubes on the basal agar medium with 5 mg. of NH_4NO_3 per tube and 0.0, 0.5, 1.0, 2.0, 5, 10 or 50 mg. of hydroxyproline. Complete or nearly complete inhibition of T-689 (A) was obtained with 1.0 mg. (1–8,000) and considerable inhibition with 0.5 mg. (1–16,000). T-689 (B) was completely inhibited by 2.0 mg. (1–4,000), markedly affected by 1.0 mg. (1–8,000) and unaffected by 0.5 mg. (1–16,000). T-689 (BB) was unaffected by 10.0 mg. (1–800) or less and inhibited slightly by 50.0 mg. (1–160), (table 3).

This demonstrated that hydroxyproline was injurious for T-689 (A), T-689 (B) and T-689 (BB) when ammonia was the nitrogen source. However, while the response of the (B) and (BB) forms was much the same whether ammonia or asparagine was used the (A) form was more susceptible to hydroxyproline with NH_4NO_3 as the nitrogen source (table 3). With NH_4NO_3 1.0 mg. (1–8,000) of hydroxyproline was sufficient completely to inhibit growth; with asparagine 1.0 mg. and 2.0 mg. of hydroxyproline markedly inhibited growth but 5.0 mg. were required for complete inhibition.

Hydroxyproline and casein hydrolysate.—The injurious effects of hydroxyproline were less pronounced in media containing peptone or casein hydrolysate than in those containing asparagine. For example, evident subsurface growth of T-689 (N) was obtained in tubes of the basal agar medium plus 1 mg. casein and 50 mg. (1–160) of hydroxyproline; 0.4 mg. (1–20,000) of hydroxyproline was sufficient completely to inhibit growth in the asparagine medium. Considerable subsurface and surface growth was obtained in tubes containing the basal agar medium supplemented with 5 mg. of casein hydrolysate and 50 mg. of hydroxyproline.

Similar results (fig. 3) were obtained with 4 other dermatophytes, furnished by Dr. Benham as follows:

Trichophyton gypseum (granular form) which was completely inhibited by 1.0 mg. (1–8,000) of hydroxyproline on the basal agar medium containing asparagine grew considerably in the same medium to which 1 mg. of casein hydrolysate was

added in the presence of 10 mg. (1–800) of hydroxyproline (fig. 3).

T. purpureum, which was completely inhibited by 5.0 mg. (1–1,600) of hydroxyproline in the asparagine medium, grew considerably on the same medium to which 1 mg. of casein hydrolysate and 10 mg. (1–800) of hydroxyproline had been added. This fungus formed no pigment in two weeks time, when grown on the asparagine medium. The addition of 1 mg. of casein hydrolysate per tube to the asparagine agar markedly increased the growth and induced the formation of pigment. Ten mg. of hydroxyproline added to the casein-asparagine medium reduced growth and eliminated the pigment.

Epidermophyton flocculosum was completely inhibited in the asparagine medium by 0.2 mg. (1–40,000) of hydroxyproline. When 1 mg. of casein hydrolysate was added to the asparagine medium growth was obtained in the presence of 10 mg. (1–800) of the amino acid. This organism also showed a marked growth response to the addition of casein hydrolysate to the asparagine medium (fig. 3).

Microsporum canis grew better on the asparagine agar plus 1 mg. of casein hydrolysate and 10 mg. of hydroxyproline than it did on the asparagine medium plus 5 mg. of hydroxyproline.

Hydroxyproline and individual amino acids.—Since casein hydrolysate overcame, at least in part, the inhibitory action of hydroxyproline, it appeared desirable to determine the effects of individual amino acids. The action of each of the fourteen amino acids which had been previously found to support growth of the (N) form of *T. mentagrophytes* was, therefore, investigated. The amino acids used were dl-alanine, d-arginine, dl-aspartic acid, d-glutamic acid, glycine, l-histidine, l-leucine, d-isoleucine, l-lysine, dl-phenylalanine, l-proline, dl-serine, l-tyrosine, dl-valine. Each amino acid was added to the basal agar medium in test tubes at the rate of 1.0 mg. per tube. Hydroxyproline was added at 0.0, 0.1, 1.0, or 5 mg. per tube.

The results with thirteen of the amino acids were similar to those obtained with the basal medium containing asparagine. Proline, however, was found, to overcome at least in part, the injurious effects of hydroxyproline. When 1 mg. of proline was added per tube nearly as good growth was obtained with 1 mg. (1–8,000) of hydroxyproline as without and some growth was obtained with 5 mg. (1–1,600) of hydroxyproline (fig. 6). Hydroxyproline at (1–8,000) completely inhibited growth when any other of the fourteen amino acids or asparagine was used as the sole source of nitrogen (fig. 6).

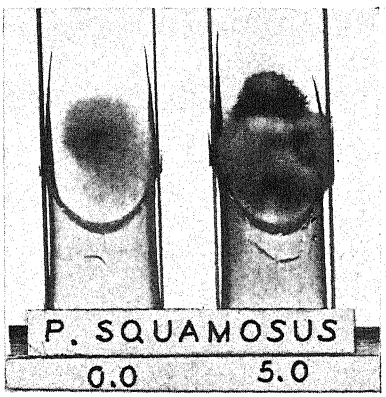


Fig. 5. *Polyporus squamosus* grown on basal agar medium plus asparagine, vitamin mixture and 1.0 mg. casein hydrolysate per tube. Left, no hydroxyproline; right, 5 mg. hydroxyproline. Age 26 days.

This suggests that the utilization by T-689 (N) of each of thirteen of the amino acids as well as asparagine was prevented by smaller amounts of hydroxyproline than necessary to interfere seriously with the use of proline by the same organism. The reduction by proline of the inhibition caused by hydroxyproline might be referred to by the term, antagonism.

The relation between proline and hydroxyproline was investigated also by measuring the dry weights produced in 2 weeks in flasks containing 10 ml. of the basal agar medium with and without asparagine. Proline was added at 0, 1, 2, 3, 5, and 10 mg. per flask. One set contained varying amounts of proline;

TABLE 3. *Inhibition of T-689 (A), T-689 (B) and T-689 (BB) on basal agar medium with NH_4NO_3 (15 days) or asparagine (13 days) as source of nitrogen and various amounts of hydroxyproline. Dash indicates given amount of hydroxyproline was not tested.*

Hydroxyproline added per tube mg.	T-689 (A)		T-689 (B)		T-689 (BB)	
	NH_4NO_3	Asparagine	NH_4NO_3	Asparagine	NH_4NO_3	Asparagine
0.2	.....	none	.....	none	....	none
0.4	.....	slight	.....	none	....	none
0.5	marked	.....	none	.....	none	....
1.0	complete	marked	marked	marked	none	none
2.0	complete	marked	complete	complete	none	none
5.0	complete	complete	complete	complete	none	none
10.0	complete	.....	complete	.....	none	none
50.0	complete	.....	complete	.....	slight	slight

a second, the proline and 5 mg. of hydroxyproline per flask. Both sets were repeated with 20 mg. of asparagine per flask.

In the media with proline as the sole source of nitrogen, growth increased with increasing proline up to those flasks containing 5 mg. of proline (fig. 7). The addition of hydroxyproline to the flasks

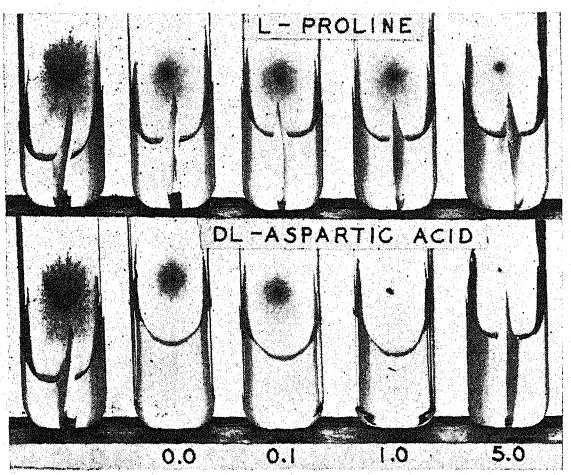


Fig. 6. T-689 (N) grown on basal agar medium. The tube on the left contains asparagine. The four tubes to the right in the upper row contain 1 mg. proline per tube; in the lower row, 1 mg. aspartic acid per tube. To the tubes containing the amino acids 0.0, 0.1, 1.0 or 5.0 mg. of hydroxyproline were added. Age 14 days.

containing proline cut the growth approximately one-half. Even 10 mg. of proline did not entirely overcome the inhibitory action of 5 mg. of hydroxyproline; in fact, judging from the shapes of the curves further increase in the proline would not have been much more effective.

Although the dry weights obtained in the asparagine-proline medium do not permit establishment of an accurate curve it appears that the addition of proline to the asparagine medium increased the dry weights. This is of interest because 20 mg. of asparagine per flask is above the maximum for increase in dry weights; further increase of asparagine would not have increased growth. In fact. the effects of the proline and asparagine appear to be more or less additive.

The addition of hydroxyproline to the asparagine medium reduced the growth from 8.3 mg. to 1.5 mg. This sample of hydroxyproline was evidently contaminated as discussed later because no growth would have occurred in the presence of 5 mg. of the more active samples of hydroxyproline. Further increase of the asparagine in the medium, as demonstrated in other experiments, would not have increased the dry weight in the presence of hydroxyproline. Proline, however, induced greater growth; 10.4 mg. dry weight of mycelium was obtained in those flasks containing 5 mg. of hydroxyproline and 10 mg. of proline. Again it appears from the general shapes of the curves that further increases in proline would not have given as good growth in the asparagine medium with hydroxyproline as without it.

Observations on the relation between proline and hydroxyproline were made also for *T. gypseum* (granular form), *T. purpureum, Microsporum canis* and *Epidermophyton flocculosum*. Each organism was grown on the basal agar medium in test tubes containing 1 mg. of arginine, phenylalanine, aspartic acid or proline. Hydroxyproline was added at 0.0, 0.2 (1–40,000), 1.0 (1–8,000) and 5 mg. (1–1,600) per tube. The response of *T. gypseum* resembled that of *T. mentagrophytes*. Proline overcame in part the inhibitory effects of the hydroxyproline; the other three amino acids did not. For *Microsporum canis* the action of the hydroxyproline was not affected by arginine or phenylalanine but was reduced by proline. Growth of this organism with aspartic acid was poor and the addition of hydroxyproline improved growth. The growth of *T. purpureum* was improved by the addition of 0.2 mg. of hydroxyproline to arginine, phenylalanine, aspartic acid and proline. These results were similar to those obtained with media containing asparagine. Larger amounts of hydroxyproline were injurious when the media contained arginine, phenylalanine or aspartic acid. Proline overcame to some extent the injurious effect of the large amounts of hydroxyproline. *Epidermophyton flocculosum* grew poorly on all four of the amino acids but the relation between them and hydroxyproline was similar to that observed for T-689 (N).

Presence of proline in samples of hydroxyproline.—It is probable that the differences in the effects on T-689 (N) of various samples of hydroxyproline were because of contamination of the samples with proline. It was found that the addition of 0.01 mg. of proline to tubes containing 1 mg. of a sample of hydroxyproline (Eastman no. 3594) which completely inhibited growth gave results

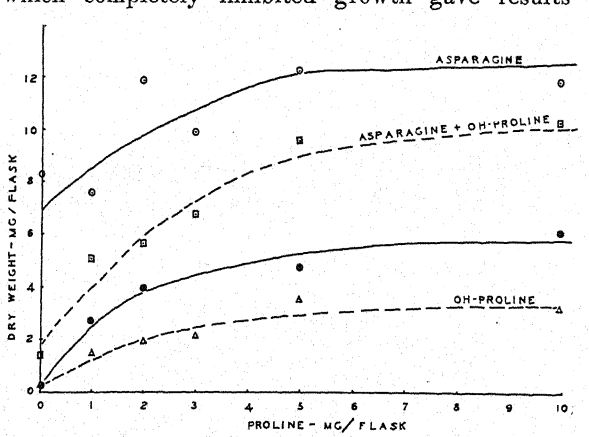


Fig. 7. Dry weight of T-689 (N) grown 14 days on basal agar medium plus 0.0, 1.0, 2.0, 3.0, 5.0 or 10.0 mg. of proline per flask and various supplements. These were, reading from the top down, 20 mg. of asparagine per flask, 20 mg. of asparagine and 5 mg. of hydroxyproline, no addition, 5 mg. of hydroxyproline.

nearly equivalent to those obtained with 1 mg. of a sample of hydroxyproline (Merck no. 42845) which permitted some growth. It appeared, therefore, that the presence of about 1 per cent of proline as an impurity in a preparation of hydroxyproline would account for the difference observed in the inhibitory action of various samples of hydroxyproline on T-689 (N) reported earlier in this paper (table 2).

In the experiments which led to the conclusion given above T-689 (N) was grown in test tubes containing the basal agar medium with asparagine. To each tube 1 mg. of a sample of hydroxyproline known completely to inhibit growth was added. Some tubes received 0.001, some 0.01 and others 0.1 mg. of proline. The beneficial effects of 0.001 mg. of proline were detectable.

Effect of methionine, tryptophane and threonine.—In the experiments on T-689 (N) previously reported from this laboratory, tryptophane, threonine, hydroxyproline, cystine, and methionine were found to be poor sources of nitrogen or to be toxic to T-689 (N). Three of these, methionine, tryptophane and threonine, were investigated further.

T-689 (N) was grown on the basal agar asparagine medium and on the basal medium plus 1 mg. of casein hydrolysate per tube. l-methionine, l-tryptophane and dl-threonine were added at 0.0, 0.1, 1 or 5 mg. per tube. The amino acids and the casein hydrolysate were autoclaved separately and added to the basal agar medium after sterilization. Tryptophane had no visible effect. Growth was reduced slightly but definitely by 0.1 mg. of methionine, but the action of 5 mg. was not much greater than that of 0.1 mg. The injurious action of methionine was as great in the presence of casein hydrolysate as in the presence of asparagine. Similar results were obtained with threonine. Although both threonine and methionine were found to be injurious to T-689 (N) under the conditions of our experiments their action differed from that of hydroxyproline. Their effectiveness increased little as the amount of the amino acid was increased from 0.1 mg. (1–80,000) to 5 mg. (1–1,600) per tube. Furthermore, casein hydrolysate, in the amount used, did not reduce the injurious action of methionine and threonine as it did that of hydroxyproline.

Propionic acid and T. mentagrophytes.—Propionic acid has been freely used as a fungistatic agent. We were interested in comparing its effect on *T. mentagrophytes* with that of hydroxyproline. T-689 (N) was grown in test tubes on the basal agar medium containing asparagine. Various amounts of propionic acid neutralized with $CaCO_3$ were added. At the rate of 0.1 mg. and 0.2 mg. per tube, propionic acid had a slight effect on early growth which disappeared later; 0.4 mg. (1–20,000) reduced growth but did not inhibit it; 1 mg. (1–8,000) or 2 mg. (1–4,000) completely inhibited growth but some tubes eventually developed a small amount of subsurface growth. In cultures containing the larger amounts of propionic acid irregularities of growth were noted. There were occasional tubes in which the organism grew much better than in others suggesting the development of pleomorphic forms resistant to propionic acid. It appeared that T-689 (N) was less susceptible to propionic acid than to hydroxyproline. In our experiments 5 mg. of propionic acid per tube reduced growth of T-689 (BB) about one-half and 10 mg. produced complete inhibition.

These results suggest that the action of propionic acid on *T. mentagrophytes* is of a different kind than that of hydroxyproline. This conclusion was supported by the fact that l-proline did not affect the toxicity of propionic acid; d-arginine and dl-valine were also found to be ineffective.

A sample of β-hydroxypropionic acid, kindly furnished us by Dr. John H. Bailey, was much less injurious than propionic acid; 1 mg. per tube (1–8,000) had no effect; with 5 mg. per tube there was some growth in 1 of 3 tubes; 10 mg. per tube gave complete inhibition.

Undecylenic acid.—The effect of a sample of Eastman undecylenic acid (9-undecylenic acid) was determined on T-689 (N), T-689 (A), T-689 (B) and T-689 (BB). Various amounts of undecylenic acid neutralized with NaOH were added to test tubes containing the basal agar medium with asparagine. Undecylenic acid was more effective than propionic acid or hydroxyproline. The addition of 0.14 mg. (1–57,000) completely inhibited T-689 (N). The (A), (B) and (BB) forms were somewhat more resistant. It required 0.4 mg. (1–20,000) completely to inhibit T-689 (A), 0.18 mg. (1–44,000) for T-689 (B) and 0.2 mg. (1–40,000) for T–689 (BB).

The action of the undecylenic acid was fungicidal. Inocula from tubes in which growth was completely inhibited failed to grow when transferred to a thiamine-peptone agar. As the threshold concentration for complete inhibition was approached the inocula in one or two tubes of three in some instances grew. When this occurred the fungus in that particular tube appeared to be little affected by the undecylenic acid even though no growth developed in the balance of the tubes in that set.

Rhodanalate and Reinecke's salt.—The possibility that contaminants of the hydroxyproline in the form of rhodanalate or Reinecke's salt might account for its inhibitory effects was considered. Samples of proline rhodanalate and of Reinecke's salt generously furnished by Dr. Max Dunn were added to the basal agar medium containing asparagine. Little or no effect was observed with 1 mg. (1–8,000) of either substance.

Other proline compounds.—We were unable to obtain a variety of proline derivatives and compare their activity with that of hydroxyproline. Merck and Company kindly supplied us with samples of γ-methyl proline and the copper salt of γ-methyl proline and Paul Lewis Laboratories with proline picrate. Methyl proline evidenced some toxicity but

much less than hydroxyproline. Little or no injury resulted from the addition of 0.2 or 0.4 mg. (1–20,000) of methyl proline to tubes of the basal agar medium containing asparagine. Slight injury was observed with 1 mg. (1–8,000) or 2 mg. (1–4,000) per tube and evident inhibition with 5.0 mg. Even 10 mg. per tube did not completely inhibit growth. The copper salt of methyl proline acted much like methyl proline though it was somewhat less effective weight for weight. Proline picrate was less injurious than methyl proline up to 5 mg. per tube but 10 mg. (1–8,000) were more inhibitory than 10 mg. of methyl proline. The injury observed with 10 mg. may have been caused by the picrate portion of the molecule.

The effect of each of these compounds on the inhibitory action of hydroxyproline also was determined for T-689 (N). All three compounds reduced

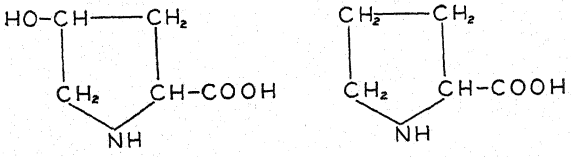


Fig. 8. Structural formulae of hydroxyproline, left; proline, right.

the injurious effects of hydroxyproline. Proline picrate was the more effective; methyl proline and its copper salt less effective.

Virulence of forms of T. mentagrophytes used.—It is generally stated in the literature that pleomorphic forms of the dermatophytes are less virulent than the normal forms. Through the courtesy of Dr. Rhoda W. Benham, inoculations of the forms we used were made on guinea pigs by scarification and rubbing in a suspension of spores. The results after 12 days were as follows:

T-689 (N)—no lesion; T-689 (A)—slight scaling; T-689 (B)—red scaling lesion about size of a quarter; T-689 (BB)—red, spreading scaly lesion about the size of a fifty cent piece.

Slides showed the fungus in the scales and invading the hair.

From these observations virulence seemed to vary directly with the degree of pleomorphism and vigor of growth of the various forms used in our experiments.

Discussion.—Although the samples of hydroxyproline we used were not especially purified, it appears that the inhibitory effects observed were caused by that amino acid and not by some contaminant.

The inhibitory effect of hydroxyproline on T-689 (N) and the other dermatophytes studied seems to be associated with the OH radical. This is evident from a comparison of the structure of hydroxyproline and proline (fig. 8). Furthermore, methyl proline was less inhibitory than hydroxyproline.

The small amount of hydroxyproline required completely to inhibit the growth of T-689 (N) and our strain of *Epidermophyton flocculosum* suggests that it may act by blocking an enzyme system. This concept might be considered to receive some support from the fact that more hydroxyproline was necessary to induce inhibition when the medium contained proline which resembles it in structure than was required for any of thirteen other amino acids.

On the other hand the beneficial effect of 0.001 mg. (1–8,000,000) of proline in the presence of 1 mg. of hydroxyproline was detectable. The activity of this small amount of proline would militate against the idea that the interrelation of proline and hydroxyproline is similar to that observed with some vitamins and their injurious derivatives. The situation is complicated because of the necessity of distinguishing between the effect of proline as a source of nitrogen and the possibility of its antagonistic action on hydroxyproline. In other words, does growth occur where proline is added to a mixture of hydroxyproline and asparagine because the organism used the proline or because proline makes the asparagine available in the presence of hydroxyproline? The results in figure 7 suggest that proline acts in the latter way because more growth was obtained in media containing asparagine, proline, and hydroxyproline than in those containing proline alone. The interrelation between proline, hydroxyproline and other sources of nitrogen requires further investigation.

A single and relatively simple explanation of the relation of hydroxyproline to the growth of fungi would not seem possible at this time. For example, let us assume that hydroxyproline interferes with growth by blocking an enzyme system. How could we explain on this hypotheses the beneficial action of small amounts of this compound on the growth of *T. purpureum?* For this organism 1 part of hydroxyproline in 40,000 parts of medium improved growth in the presence of arginine, asparagine, aspartic acid, phenylalanine or proline while larger amounts (1–4,000) inhibited growth. A small amount of hydroxyproline appeared to increase the ability of *T. purpureum* to use these various sources of nitrogen since we found that in the absence of any other source of nitrogen it had no stimulating effects. Furthermore, it would be difficult to explain on the hypothesis suggested why the growth of *Polyporus squamosus* was unaffected by small amounts of hydroxyproline while larger amounts (1–1,600 markedly increased its growth.

The difference in the relation of the dermatophytes which have been investigated and other fungi to hydroxyproline suggests some physiological characters common to this group. What these may be we cannot say with certainty. It seems probable that the action of hydroxyproline is associated in some way with the nitrogen metabolism of these organisms. This is suggested by the greater sensitivity of the normal form of *T. mentagrophytes* to hydroxyproline as compared to the pleomorphic forms. The latter, which are the more resistant, are

also better able to supply their nitrogen requirements from inorganic nitrogen or from a single source of organic nitrogen.

The susceptibility of T-689 (N) to hydroxyproline might suggest the use of this compound as a therapeutic agent in the control of infection. However, the development of virulent forms of the fungus resistant to hydroxyproline eliminates that possibility. The question as to whether derivatives of proline other than hydroxyproline would be more effective remains open.

In any event, it is clear that the relation of amino acids to the growth of *T. mentagrophytes* cannot be covered by a simple generalization. Some of them serve as sources of nitrogen, some are unavailable, some are inhibitory and their effects when two or more are present together depend upon which amino acids are used.

Several investigators have reported the inhibitory effects of specific amino acids on other organisms and in some instances the antagonism of these effects by various amino acids.

For example, Gordon and McLeod (1926) reported cystine, glycine, phenylalanine and tryptophane inhibitory to various cocci and suggested that the deamination products might be the actual inhibitors. Gladstone (1939) found leucine, threonine, and α-amino-butyric acid inhibited *B. anthracis*. Valine overcame the inhibition. The inhibition produced by isoleucine, norleucine and serine was relieved by valine and leucine and the serine inhibition by threonine. The effective quantities of the amino acids were minute and Gladstone suggested that they were involved in competition for enzymes. Bonner, Tatum and Beadle (1943) found phenylalanine, norleucine or norvaline inhibited the activity of isoleucine and valine for a mutant Neurospora requiring isoleucine and valine. Doermann (1944) studied the inhibition by arginine of a lysineless mutant of *Neurospora*. This inhibition was antagonized by lysine. Greene (1945) reported the growth of *Leptospira canicola* to be decreased by alanine, arginine, glutamic acid, glycine, lysine, methionine, phenylalanine, tryptophane, tyrosine or valine.

It appears that the effects of specific amino acids vary widely with the organisms concerned and we do not yet have satisfactory explanations for the action of some of them.

SUMMARY

The growth of *T. mentagrophytes* on a basal medium containing minerals and dextrose with asparagine as a source of nitrogen was inhibited by 1 part of l-hydroxyproline in 20,000 parts of medium. Two pleomorphic forms were less susceptible and a third grew in the presence of 1–160 hydroxyproline. The inhibitory effects of hydroxyproline were overcome in part by l-proline but not by any one of thirteen other amino acids. *Trichophyton gypseum* (granular form), *T. purpureum, Epidermophyton flocculosum* and *Microsporum canis* were also inhibited by hydroxyproline though they differed in sensitivity. Growth of *Trichophyton purpureum* was stimulated by small amounts of hydroxyproline and inhibited by larger amounts. Quantities which inhibited the five dermatophytes did not act injuriously on nineteen other fungi, including species of *Agaricus, Aspergillus, Ceratostomella, Fomes, Neurospora, Penicillium, Phycomyces, Polyporus* and *Rhodotorula*. Growth of *Polyporus squamosus* was improved by hydroxyproline. The action of hydroxyproline on *T. mentagrophytes* was fungistatic. The effects of propionic acid, β-hydroxyproline acid, undecylenic acid, rhodanalate, Reinecke's salt, methionine, l-tryptophane and dl-threonine on *T. mentagrophytes* are reported briefly. Methyl proline, the copper salt of methyl proline and proline picrate were less inhibitory than hydroxyproline. The pleomorphic strains of *T. mentagrophytes* were found to be virulent for the guinea pig.

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SEX DETERMINATION AND SEX BALANCE IN MELANDRIUM [1]

H. E. Warmke

CAPACITIES FOR male development or for female development are established at fertilization in those animals and higher plants with separate sexes and depend on the chromosome constitutions of the participating gametes. Among the mammals and most dioecious plants (XX female; XY male) the female produces gametes of only one kind, those with an X chromosome; but the male produces two kinds of gametes, those with an X chromosome and those with a Y chromosome, in approximately equal numbers. Each zygote thus receives an X chromosome from the female parent; if it also receives an X from the male parent it becomes female (XX), but if it receives a Y from the male parent it becomes male (XY). Chromosomes are likewise known to provide the differentiating mechanism in other types of sex inheritance: in the XO type (Orthoptera), where the female has two sex chromosomes (XX) and the male only one (XO), and among the birds and Lepidoptera, where the female commonly is heterogametic (XY or XO) and the male homogametic (XX).

Sex chromosomes probably act in sex differentiation by so altering the proportion between male and female determiners that one sex or the other results. For example, in the XO type (male heterogametic) the autosomes must have a predominant male influence and the X chromosome a predominant female influence, these being so balanced that maleness results when the autosomes are opposed by a single X chromosome, but that femaleness results when they are opposed by two X chromosomes. In the XY types also there is a difference in the proportion of sex genes in the XX and XY individuals. In this case, however, it is not possible by inspection to say which chromosomes bear the respective male and female tendencies. Here males and females have the same number of chromosomes, and at least three possible distributions of the sex genes must be distinguished:

	Female	Male	Critical ratio	Example
	♀♀	♀♂		
(1)	AAXX	AAXY	X/Y	*Melandrium dioicum*
	♂♂♀♀	♂♂♀		
(2)	AAXX	AAXY	X/A	*Drosophila melanogaster*
	♀♀	♀♀ ♂		
(3)	AAXX	AAXY	A/Y	*Platypoecilus xiphidium*

The first of these is probably a primitive condition, with the opposing sex genes in homologous or partly homologous chromosomes (X and Y) and segregating to determine the two sexes. This distribution is known to exist in *Melandrium* and will be described more fully in the present paper. The second case, in which the X/A ratio is critical, is known to exist in both plant and animal species (*Rumex acetosa* and *Drosophila melanogaster*). This type may well have been derived from the first through the loss of activity of the Y chromosome (Muller, 1932a). The third is not likely to be a common type, but it has been reported for the fish, *Platypoecilus xiphidium,* by Kosswig (1936a).

There are, theoretically, also three complementary possibilities for the distribution of the sex determiners if the female is heterogametic, but these need not be considered in detail here. It is also possible that a stable sex mechanism may have evolved in which either the male or the female determiners are distributed in more than one location; for example, the autosomes might have a weak overall male influence which would induce maleness only when operating with a weakly male-determining Y chromosome.

It is well to have these various possibilities for the distribution of the sex factors clearly in mind, because they have not always been recognized or critically distinguished in the past. There has been a tendency on the part of many workers to assume a common plan for the distribution of the sex-determining genes in all organisms. Frequently this has been the one most clearly understood, that of *Drosophila.* In other instances the presence of a Y in XY individuals has caused investigators to ascribe a positive effect to the Y which as far as altering sex balance is concerned, might equally well have been due to the concomitant absence of an X chromosome. Other investigators, though recognizing the alternate possibilities for the locations of the sex determiners, have nevertheless espoused particular theories without really critical evidence. This has been particularly true in certain cases where conclusions have been based on genetic evidence, unaccompanied by cytological studies.

Bridges (1922, 1925, 1939) was probably the first to clearly point out methods of placing the sex genes in particular chromosomes. He worked on *Drosophila melanogaster* and studied triploids and various nondisjunction types, in which the ratios of X and Y chromosomes, and of sex chromosomes and autosomes, could be altered. Table 1 gives a list of the chromosome types he studied in this now classic work.

The X chromosome here obviously is female-determining; 2A X individuals are male, 2A XX female, and 2A XXX superfemale. The Y chromosome, on the other hand, plays no positive role in sex determination; it apparently contains no important sex determiners. Maleness results whether the Y is present, as in 2A XY individuals, or absent, as in 2A XO individuals. The only importance of the Y, apparently, is to contribute fertility genes, the XO males being anatomically normal but ster-

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ile (Stern, 1929). The male determiners are carried in the autosomes, 2A XX individuals being normal females and 3A XX intersexual. Sex is determined in this species, therefore, by the ratio of number of X chromosomes to number of sets of autosomes. An X/A ratio of 1.0, or higher, produces femaleness, a ratio of 0.5, or lower, produces maleness, with the intermediate ratios of 0.67 and 0.75 resulting in intersexuality; and there can be little question of the validity of these conclusions, based as they are on observation of the effects of adding or removing X and Y chromosomes and of altering the ratios of sex chromosomes and autosomes.

The location of the sex tendencies has been determined also for *Rumex acetosa,* by a number of Japanese workers (summarized by Ono, 1935, and Yamamoto, 1938). Using the same methods as Bridges, and studying sex in a large number of balanced and unbalanced polyploid types, these workers were able to show quite conclusively that the distribution of sex factors in *Rumex* is similar to that in *Drosophila.* Their observations are in agreement with a scheme which allocates a net male tendency to the autosomes, a net female tendency to the X chromosomes, and no influence to the Y chromosome. It is of further interest that Yamamoto (1938) has been able to show that, while the total effect of the autosomes in *Rumex* is towards maleness, certain autosomes (a2 and a5) actually have slight overall female tendencies.

Doncaster's studies on *Abraxas grossulariata* (1914 and earlier), although not completely interpreted at that time, seem to furnish sufficient evidence for the location of the sex genes. Males and females normally have 56 chromosomes in this species, but a race was discovered in which females had only 55 chromosomes. Since the female is known to be heterogametic, from studies of sex-limited inheritance, this strongly suggests that XY individuals (56 chromosomes) and XO individuals (55 chromosomes) both are female and therefore that the Y chromosome carries no important sex factors. This, further, would immediately place the female factors in the autosomes and the male factors in the X chromosomes.

The evidence for the location of the sex-determining genes in fishes is not supported by crucial cytological studies, and therefore is not so clear-cut as where such studies are possible. Winge has maintained from the beginning of his studies (1922, 1923) that a dominant male sex factor is present in the Y chromosome and a recessive female sex factor in the X chromosome of *Lebistes reticulatus.* He does not appear to have distinguished critically, however, between the presence of a Y and the absence of an X in reaching these conclusions. More recent studies (Winge, 1934), of one race in which a pair of autosomes has taken over the role of sex chromosomes, giving XX males and females, and of another in which XY individuals are female, are interpreted as indicating that numerous sex factors, both for maleness and for femaleness, are present in the autosomes, and therefore, that sex chromosomes differ from autosomes only in possessing stronger sex-determining genes.

TABLE 1. *Chromosome constitution and sex in Drosophila (After Bridges).*

Chromosome constitution	Sex	X/A ratio
2A XXX	Superfemale	1.5
AX (patches)	Female	1.0
2A XX		
2A XXY		
3A XXX		
4A XXXX		
3A XX	Intersex	0.67
3A XXY		
4A XXX	Intersex	0.75
2A X	Male	0.50
2A XY		
2A XYY		
4A XX		
3A X	Supermale	0.33

Kosswig (1936a, b), also working on fishes, has studied the location of the sex factors in *Platypoecilus maculatus* and *P. xiphidium.* These species, though capable of being crossed, have opposite sex-determining mechanisms: in *maculatus* the female is heterogametic, and in *xyphidium* the male is heterogametic. By making appropriate interspecific crosses and following the sex chromosomes by means of marker genes, Kosswig has been able to study the effects of X, Y, and autosomes on sex determination. He concludes that the X chromosomes lack sex factors in both species, but that the autosomes are male-determining and the Y female-determining in *maculatus,* while the autosomes are female-determining and the Y male-determining in *xyphidium.*

Goldschmidt believes that in *Lymantria* (female XY, male XX) the male determiners are located in the X chromosome, the female determiners are inherited maternally, and the autosomes contain only secondary modifiers for femaleness. The maternal inheritance of femaleness he has ascribed alternately to the Y chromosome and to the cytoplasm. He decided in favor of Y-borne female factors when, from the cross of a weak female with a strong male, exceptional, strong females were obtained—apparently by receiving a Y chromosome through nondisjunction from a strong XXY father (Goldschmidt, 1922). This hypothesis was abandoned in favor of a cytoplasmic male influence, when no all-male broods appeared after females from crosses between sex-reversed XY males and normal XY females were mated to strong males (Goldschmidt, 1934). After the demonstration of male-determining factors in the Y chromosome of *Melandrium* (Warmke and Blakeslee, 1939b), Goldschmidt (1942) called attention to possible loopholes in the cytoplasmic explanation and is again inclined to regard the Y as the bearer of the female factors.

In the liverwort *Sphaerocarpos Donnellii* there is some question about the location of the sex factors. Allen long has argued for X-borne female factors and Y-borne male factors: (1) because of the simplicity of this explanation in fitting the facts observed in the bryophytes (Allen, 1932, 1935b), and (2) because, while 2A XY gametophytes (secured by germination of spore dyads) are female with certain inter-sexual tendencies, 2A XO gametophytes have been observed to be strictly female (Allen, 1935a; Mackay and Allen, 1936). He recently has suggested, however, that the genetics of sex in the bryophytes may be more complex than the early observations indicated (Allen, 1945). Knap (1935a, b) X-rayed sporophytes of *Sphaerocarpos* during meiosis and obtained tetrads which produced three males and one female, instead of the usual two males and two females. A cytological analysis of these tetrads showed that two of the males were AY, but that the third male was AX or A plus a fragment of an X, with no trace of a Y chromosome. This is interpreted as indicating that the X chromosome contains female factors which may be inactivated or lost through the action of X-rays, and that, when this occurs, male genes in the autosomes express themselves to produce a male gametophyte without the presence of a Y chromosome. Lorbeer (1941) believes, as Allen, that the X chromosome is female-determining and that the Y is male-determining in *S. Donnellii.* He, however, postulates a single gene pair as responsible for maleness or femaleness. The results of the irradiation studies are interpreted as being due to the mutation of the female gene in the X to an intermediate allel, or directly to the male allel, rather than to loss or inactivation of the female genes.

In the addition to his work on *Lebistes,* Winge (1931) has also made some studies on *Melandrium,* and he concludes that sex in this species "is determined by the strength of sex genes in the different X's and Y's, and furthermore by autosomal genes, pulling in male or female direction." In addition he has studied the inheritance of four sex-linked characters, "auria" (Xau), "abnormal" (Xn or Yn), "variegated" (llmmoo Xn Xn), and "hermaphroditic males" (Xf). Inasmuch, however, as it was necessary to postulate the presence of at least nine inhibitors or lethals to explain the observed facts regarding the inheritance of these four characters, and since Winge assumes that "the difference in size and form of the X and Y in *Melandrium* is very pronounced, the X being about twice as large as the Y," which has since been shown to be incorrect (Warmke and Blakeslee, 1939b), perhaps these interpretations should not be taken as final.

Studies of sex in the higher plants were undertaken in this laboratory early in 1938. First- and second-generation tetraploids in *Melandrium, Spinacia,* and *Humulus* were reported the same year (Blakeslee, Warmke, *et al.,* 1938), as were preliminary interpretations of results (Warmke and Blakeslee, 1939a). Somewhat later (1939b) it was possible to report the sex of $3n$ and $4n$ populations and of various aneuploid types in *Melandrium.* The association of the sex chromosomes in 4A XXYY plants was described, the establishment of a $4n$ dioecious race was discussed, the Y chromosome was demonstrated to be larger than the X, and it was shown that the Y chromosome contains strong male-determining elements. The cross 4A XXXX by 4A XXXY was accomplished, and led to the production of a race giving approximately equal numbers of males and females (Blakeslee, Warmke, *et al.,* 1939). In December of the same year the Y chromosome was noted to be male-determining, the X chromosome female-determining, and the autosomes unimportant in primary sex determination (Warmke and Blakeslee, 1940a). Further evidence for the location of the sex-determining factors was presented late the following summer (1940b), as well as data on investigations other than the location of the sex factors (1940c).

Westergaard undertook a study of the sex mechanism in *Melandrium* without any knowledge of the work done in this laboratory (*cf.* Westergaard, 1940) and published a preliminary note on first-generation tetraploids (November 19, 1938), a few days before the first report from this laboratory appeared (December 9, 1938). This early report of Westergaard's was the first knowledge our laboratory had that work of this nature was under way in Denmark. In the 1938 paper Westergaard announced the production of first-generation tetraploids, 6 males (XXYY) and 4 females (XXXX), by heat and by colchicine treatment. He also described the pairing of the sex chromosomes at meiosis in the tetraploid males, and predicted an excess of 4A XXXY plants in the second generation. He mentioned that $4n \times 2n$ and reciprocal crosses were possible in *Melandrium,* but still considered the X chromosome to be larger than the Y. In 1940 Westergaard published a very comprehensive account of his excellent studies on *Melandrium* (nine chapters, 131 pages). In this work he confirmed the strongly male-determining nature of the Y chromosome and the fact that the Y is larger than the X. Regarding the location of the sex-determining factors, Westergaard concluded: "The sex in *Melandrium* is determined normally by a balance between a very strong male determining element in the Y and a female determining element, distributed in the X chromosome and in the autosomes. Moreover at any rate some of the autosomes must contain male determining genes." This is in part at variance with the results obtained from American stocks (Warmke and Blakeslee, 1939b, 1940a, b), which indicated that the Y chromosome is male-determining, that the X chromosome is female-determining, and that the autosomes play little if any role in primary sex determination.

Ono also has studied sex in *Melandrium* by means of experimental polyploidy. He has observed the

conjugation of the sex chromosomes in tetraploid males, has secured triploid seedlings, has ascertained that the Y is larger than the X (Ono, 1939a, b), and has found by the study of a series of polyploid and aneuploid types that the Y chromosome carries male-determining factors (Ono, 1940), thus confirming the earlier observations made in this country.

Löve (1944), in a study aimed at elucidating the taxonomic relationship of the red- and white-flowered forms of *Melandrium,* also has made some observations on localization of the sex genes. She agrees that the Y is male-determining, but follows Westergaard in believing that the female tendency is divided between the X and the autosomes. Two reports from this laboratory (Warmke and Blakeslee, 1940a, b) apparently have not been available to her.

It is the purpose of the present paper to give complete evidence, from experiments carried on at this laboratory during the past several years, for the location of male- and female-determining genes in *Melandrium,* and for an estimation of the relative strength of these genes. The publication of these data has been delayed because of this laboratory's preoccupation with war research in other fields.

Materials and methods.—The standard white-flowered stock of *Melandrium dioicum* (Warmke and Blakeslee, 1940c) was used throughout (*Melandrium dioecum,* ssp. *album,* according to Löve, 1944). Polyploidy was induced by soaking seeds in aqueous solutions of colchicine (0.05 to 0.4 per cent) for periods of 1 to 4 days.

Plants with new ratios of sex chromosomes, and of sex chromosomes and autosomes, were secured in the following ways: (1) By selecting nondisjunction types, which lack or have an excess of specific individual chromosomes—nondisjunction of sex chromosomes and autosomes being relatively common in autopolyploids of Melandrium. (2) By crossing triploids with tetraploids and with diploids, and by selecting plants with desired chromosome constitutions from the resulting heterogeneous progenies. (3) By crossing various polyploid plants from which euploid types with new ratios of sex chromosomes (XXY, XXXY, etc.) were produced by segregation and recombination. These new types have made it possible to study, separately, the roles of the X chromosome, of the Y chromosome, and of the autosomes in sex determination.

In *Melandrium* it is possible to determine the sex-chromosome constitution of a plant by cytological means. Y chromosomes are larger than X chromosomes, which in turn are larger than any of the eleven pairs of autosomes (fig. 1). Measurements of somatic chromosomes from root tips indicate that the Y is approximately 1.6 times the length of the X, and that the X is about 1.5 times longer than the longest autosome (Warmke and Blakeslee, 1940c). Both X and Y chromosomes have median or submedian centromeres.

The chromosomes from root tips were prepared for study by the section-smear technique (Warmke, 1941), using Belling's modified Navashin fluid, or Benda's fluid and the Feulgen stain. The most satisfactory preparations resulted when root tips ½ to ¾-inch in length were removed from a plant, washed, placed in a refrigerator at 0°C. for 1½ hours in small vials of spring water, and then killed in cold Benda fluid. The pre-treatment at 0°C. tends to shorten the chromosomes and thus to prevent overlaps, while the Benda fluid preserves the centromere constrictions (Warmke, 1946). Meiosis was studied in the anthers of males and hermaphrodites from aceto-carmine smear preparations. Buds were killed and fixed overnight in 3:1 absolute alcohol and acetic acid. The next morning the killing fluid was removed and replaced with a few drops of aceto-carmine, which was allowed to act for another 24 hours or so. The anthers were then crushed out in a drop of aceto-carmine on a slide, covered with a small coverslip, pressed, sealed, and studied as temporary mounts. Propionic acid may be substituted for acetic in both killing fluid and stain with equally good or superior results. The chromosome constitutions of female plants have been determined largely from root-tip preparations, because of the technical difficulties of studying the divisions in the macrospore mother cell. When these divisions were studied (4A XX type), ovaries were killed in Navashin fluid, sectioned, and stained by the Feulgen method.

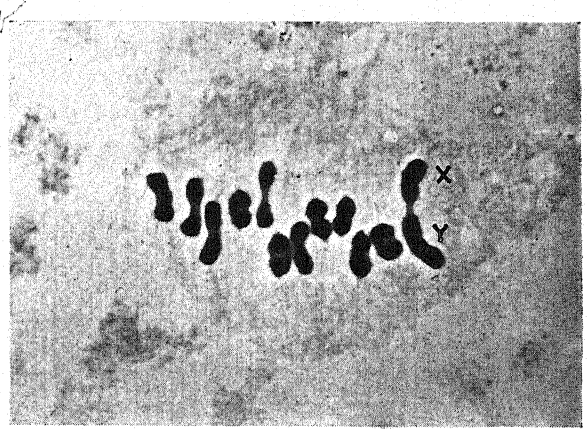


Fig. 1. Photomicrograph of chromosomes at first meiotic metaphase in diploid male of *Melandrium,* showing 11 autosomal bivalents and heteromorphic XY pair, ×1400.

Observations.—*Role of the Y chromosome in sex determination.*—When diploid males (2A XY) and diploid females (2A XX) are treated with colchicine, they become tetraploid males (4A XXYY) and tetraploid females (4A XXXX) respectively; and when these tetraploid individuals are intercrossed, an F_1 population is produced

which consists of approximately 3.5 per cent 4A XXXX (females), 3.5 per cent 4A XXYY (males), 89 per cent 4A XXXY (males and male-hermaphrodites), and 4 per cent other types having three or five sex chromosomes (Warmke and Blakeslee, 1939b, 1940c). The first two classes reconstitute the parental types, but the third and largest class, 4A XXXY, is a new combination. These XXXY plants, in spite of the altered ratio of X and Y chromosomes, are almost exclusively male and gen-

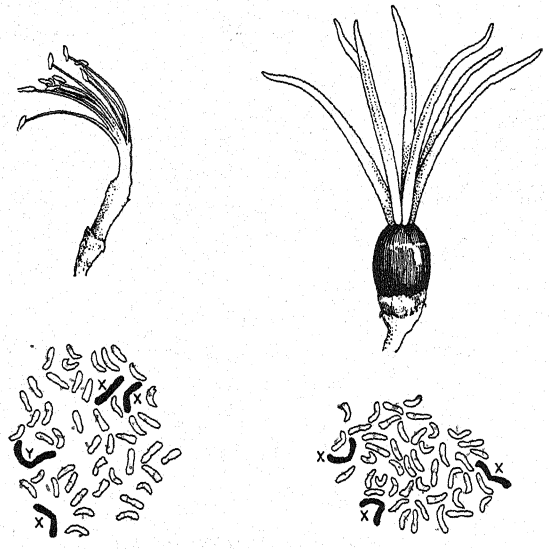


Fig. 2. Flowers and camera lucida drawings of somatic chromosomes from 4A XXXY and 4A XXX plants of *Melandrium*. These plants differ cytologically only in that one has a Y chromosome, which is absent in the other; this causes one to be male and the other female.

erally indistinguishable vegetatively from the XXYY males.

Among the plants with three or five sex chromosomes, which result from nondisjunction of the sex chromosomes, one plant was found that had four sets of autosomes but only three X chromosomes (4A XXX). This plant was female (Warmke and Blakeslee, 1939b). When 4A XXXY and 4A XXX plants are compared with regard to chromosome constitution, it is evident that they differ solely in that the former (fig. 2, left) possesses a Y chromosome while the latter does not (fig. 2, right). The 4A XXXY plant, however, is male, while the 4A XXX is female. This indicates that the Y chromosome plays a definite role in sex determination in *Melandrium*: that it contributes a male tendency strong enough in this case to change the balance from femaleness to maleness. Subsequently this three-X female was crossed to various tetraploid males, and twelve more plants with the same chromosome constitution were obtained; all were female.

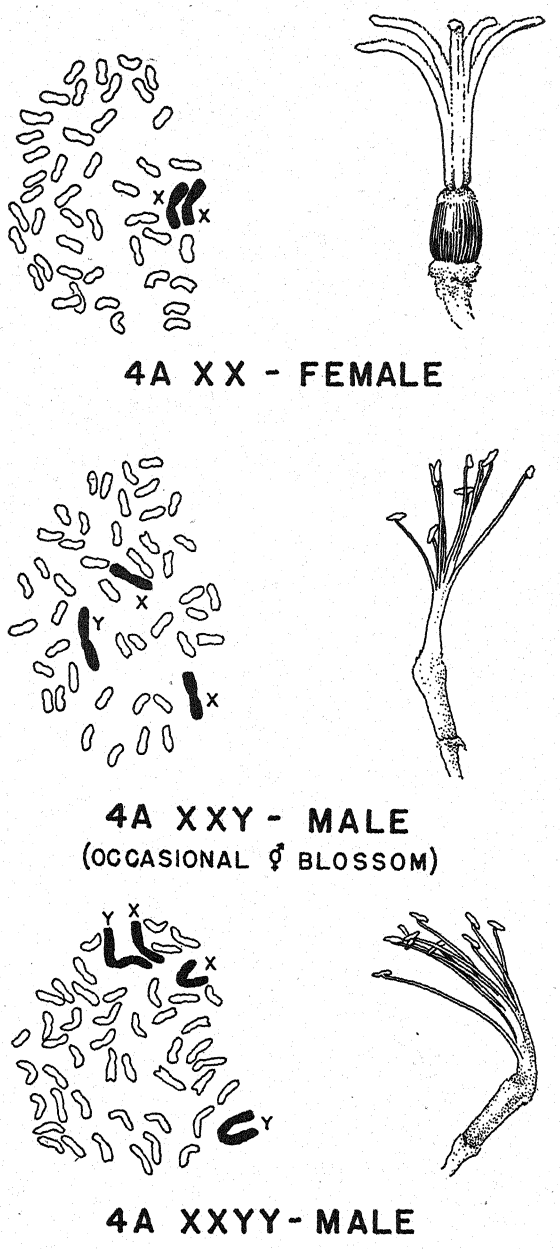


Fig. 3. Series indicating the presence of male determiners in the Y chromosome. All plants are similar in having four sets of autosomes and two X chromosomes. They differ in the number of Y chromosomes, which change the sex from complete femaleness to complete maleness.

More evidence about the role of the Y chromosome is provided by the series of plants illustrated in figure 3. Each of these has four sets of autosomes and two X chromosomes; the only variable is the number of Y chromosomes. The plant at top of the series has two X's and no Y, and is female. The next plant has two X chromosomes plus one Y chromosome, and is almost completely male, although plants of this constitution show some un-

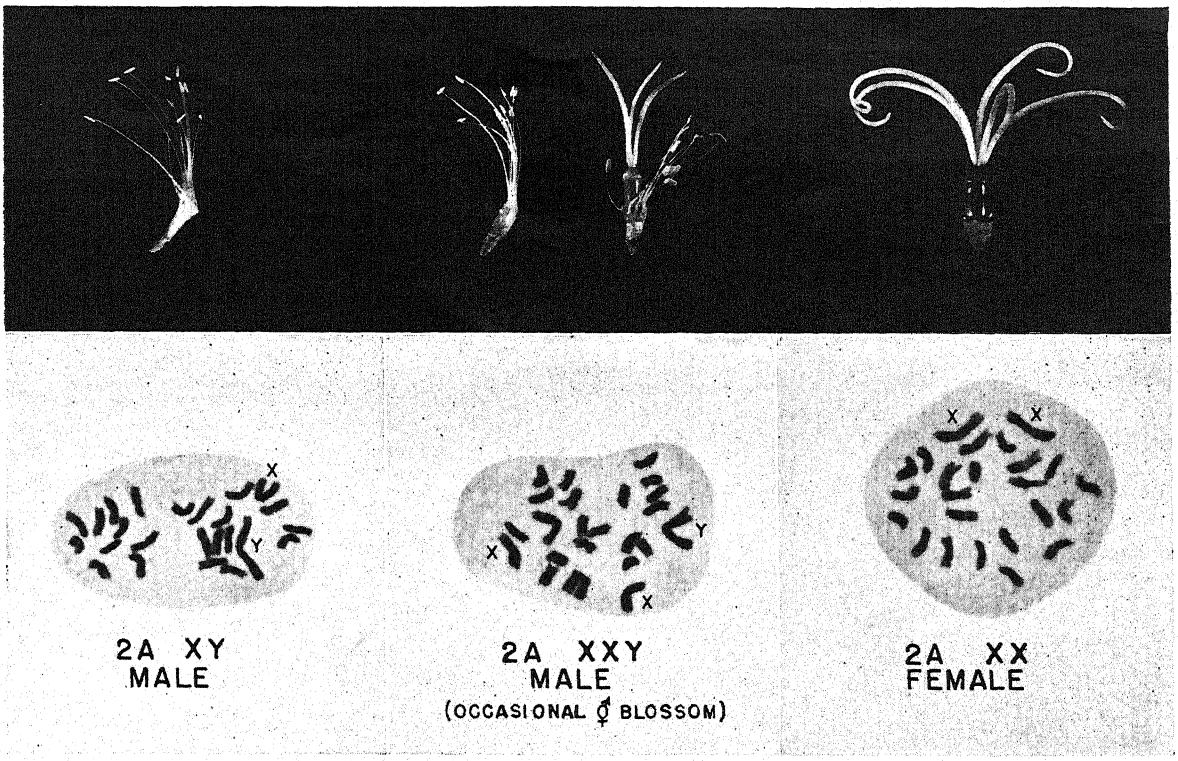


Fig. 4. Photographs of dissected blossoms, ×1, and photomicrographs of root tip chromosomes, ×1400, of 2A XY (male), 2A XXY (male-hermaphrodite), and 2A XX (female) plants of *Melandrium*.

balance by occasionally producing blossoms with more or less well-developed pistils. The plant at the bottom of this series has two X's and two Y's, and is strictly male. Each of these three types has appeared in our cultures many times, and in different stocks; there is reasonable assurance, therefore, that they are representative. In this series the addition of one Y chromosome causes a strong shift towards maleness, but the addition of two Y's results in complete maleness. Sex is thus changed from complete femaleness to complete maleness through the action of two Y chromosomes.

Further evidence of potency of the Y chromosome is provided by another nondisjunction plant, 4A XXXXY. This plant with four X's and one Y is strongly hermaphroditic (fig. 5, bottom row). It differs chromosomally from the normal 4*n* female (4A XXXX) only in having a Y chromosome. This Y chromosome expresses itself by producing stamens in the 4A XXXXY blossoms; stamens have never been found on 4A XXXX plants.

Westergaard has found a plant of this same constitution (4A XXXXY) among his tetraploids, but his plant is strictly male (Westergaard, 1940). It seemed desirable, therefore, to re-examine our original case to determine whether it was abnormal in any way, possibly with a deficient Y chromosome which permitted the development of hermaphroditism. In root tips the Y appeared to be normal, with arms of approximately equal length, and at meiosis it was associated with the X's in the various expected configurations, thus showing no cytological abnormality. As a further check on the character of the XXXXY plant, it was selfed and also outcrossed to an XXXY male, and the offspring were studied cytologically. The self yielded 7 females (6 XXXX and 1 XXXXX), 5 males (2 XXXY, 2 XXXYY, 1 XXXXYY), and 8 hermaphrodites (XXXXY). The cross produced 9 males (1 XXYY, 5 XXXY, 3 XXXYY), 6 females (XXXX), and 4 hermaphrodites (XXXXY). This is good evidence that the Y chromosome was not abnormal in our original hermaphrodite; for without exception in these new combinations (cross as well as self), when the Y was opposed by four X's the resulting plant was strongly hermaphroditic (fig. 5, bottom), but when it was opposed by only three X's the resulting plant was male, as expected. (Some plants of this latter type might have been expected to be weakly hermaphroditic, judging from our past experience with XXXY plants; but in this instance none appeared.) It would seem, therefore, that the 4A XXXXY type in our stocks is regularly hermaphroditic, and in this respect differs from the plant observed by Westergaard.

Two of the plants shown in figure 4 likewise indicate activity on the part of the Y chromosome. 2A XX plants (fig. 4, right) are normal diploid females; when a Y chromosome is added the sex of the resulting plant is almost completely reversed.

The 2A XXY plant (fig. 4, center) produces mostly male blossoms, but shows sufficient unbalance to produce an occasional blossom with both male and female reproductive structures.

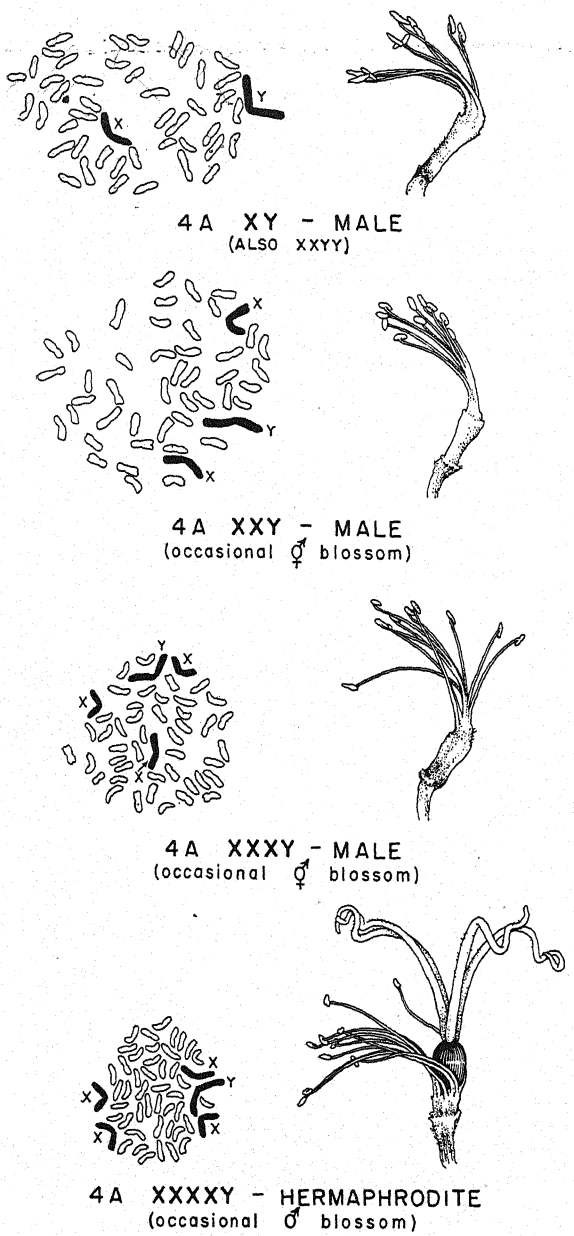


Fig. 5. Series showing the female-determining tendency of the X chromosome. All plants have four sets of autosomes and one Y chromosome, but they differ in the number of X chromosomes. As the number of X's increases, femaleness increases.

Thus, in four critical cases, the Y chromosome is found to have a strong male tendency; there can be little doubt, therefore, that the Y chromosome plays an active role in the determination of sex in *Melandrium*.

Role of the X chromosome in sex determination.—It is likewise possible to select, from spontaneous nondisjunction types among the various polyploids, a series of plants which indicates the role of the X chromosome in sex determination. Figure 5 shows such a series. Here four types of plants have been selected, all having four sets of autosomes and one Y chromosome, but differing in the number of X chromosomes. Any difference in the sex of plants in this series, therefore, must result from the action of the different numbers of X chromosomes.

Plants with one X and one Y (also XXYY plants, which have the same X/Y ratio) are male (fig. 5, top). No exception to this has been observed. Plants that have two X's to one Y are also strongly male, although rare blossoms with a poorly developed pistil (in addition to stamens) have been observed. The 4A XXXY plants are predominantly male but occasionally do produce some hermaphroditic flowers. When one Y is opposed by four X's, however, hermaphroditic blossoms almost always result. Thirteen plants of this type have been obtained; all the strongly hermaphroditic and only very occasionally is a completely male blossom observed.

From the XY plants, which are strictly male, to the XXXXY plants, which have about equally well-formed male and female structures, there is a tendency towards an increase in femaleness as the number of X chromosomes is increased. This indicates the presence of female-determining genes in the X chromosome.

The pedigrees described in the previous section, in which 4A XXXY and 4A XXXXY plants were obtained as segregates from the same self or cross, may also be considered in connection with the role of the X chromosome. The first type (XXXY) is strongly male, and rarely produces a blossom with a sufficiently well-developed pistil to set a capsule and seed; while the second (XXXXY) is strongly hermaphroditic, with almost every blossom setting a well-developed capsule of seed.

Further evidence for the activity of the X chromosome may be adduced from the plants in figure 4. The plant in the center (2A XXY) differs chromosomally from that on the left only in having an additional X chromosome. This X chromosome evidently exerts a female influence, because the XXY plants clearly have augmented female tendencies.

The occurrence of several spontaneous breaks in the X chromosome, involving the loss of an entire or almost an entire arm, has made it possible to locate the female tendency more exactly, in one arm of the X chromosome. Among the offspring of 2A XXY plants, individuals have appeared on several occasions which are hermaphroditic to about the same degree as the parent but which have a fragmented X chromosome. The fragment appears to be approximately one-half the length of a normal X chromosome and to have a terminal, or very nearly terminal centromere, thus indicating that

one complete arm or almost the complete arm has been lost. The remaining centric fragment associates in a high percentage of cases with the intact X chromosome, and never with the Y chromosome. This indicates that the X-fragment consists of the arm not homologous to the Y, or the so-called differential arm of the X. Since this fragment seems to be as effectual in inducing hermaphroditic blossoms as the entire X chromosome of XXY plants, it appears that the female tendency of the X chromosome is localized in this differential arm.

Role of the autosomes in sex determination.—It seemed possible that the autosomes also might play a part in sex determination in *Melandrium*, as they are known to do in *Drosophila* and *Rumex*. To test this possibility a series of plants was selected in which the sex-chromosome constitution was the same (XXY), but in which the number of sets of autosomes was altered (2A, 3A, 4A, fig. 6). XXY is a somewhat labile condition for the sex chromosomes, being between XY and XX in balance; therefore, any important effect of the autosomes, in the direction of either maleness or femaleness, should be observable. As is shown in figure 6, no essential change in sex is observed as the number of sets of autosomes is increased; all three types produce a large majority of male blossoms, with an occasional hermaphroditic blossom. Any important effect of balanced sets of autosomes, therefore, seems to be ruled out.

Table 2 shows chromosome constitution, X/A ratio, and sex, of a series of plants with an extreme range in X/A ratio. The first plant, for example, has four sets of autosomes and only two X chromosomes, or an X/A ratio of 0.5. The last one has two sets of autosomes and three X chromosomes, or an X/A ratio of 1.5. The others are intermediate and form a complete series between these extremes. If balanced sets of autosomes carry any appreciable male influence, it should be expressed here. However, with a range in X/A ratio of three—from 0.5 to 1.5—there is no effect on sex; all plants are female.

The first plant in table 2 is of special interest; though strictly female, it has the same X/A ratio (0.5) as the diploid *Melandrium* male (2A XY). This may be taken to indicate that the X/A ratio is not critical in sex determination in *Melandrium*,

TABLE 2. *Relation of X chromosome-autosome (X/A) ratio to sex in Melandrium.*

Chromosome constitution	X/A ratio	Sex
4A XX	0.5	Female
3A XX	0.67	Female
4A XXX	0.75	Female
2A XX, 3A XXX, 4A XXXX	1.0	Female
4A XXXXX	1.25	Female
2A XXX	1.50	Female

and therefore that automosomes, in balanced sets, have no appreciable male effect.

More evidence for the lack of importance of the autosomes in sex determination in this species is afforded by a study of plants resulting from crosses involving a triploid, either $3n \times 3n$, $4n \times 3n$, or $3n \times 4n$. These data were presented in another connection as tables 4, 5, and 6 of a previous publication (Warmke and Blakeslee, 1940c). In these crosses almost a complete array of types of plants having from 25 to 48 chromosomes—including two, three, and four sex chromosomes—appeared. If some of the individual autosomes do carry male or female sex genes, these might be expected to show their influence here, under varying conditions of autosome and sex-chromosome unbalance. From these pedigrees, however, there appeared only four plants, from among two hundred studied, that produced any hermaphroditic

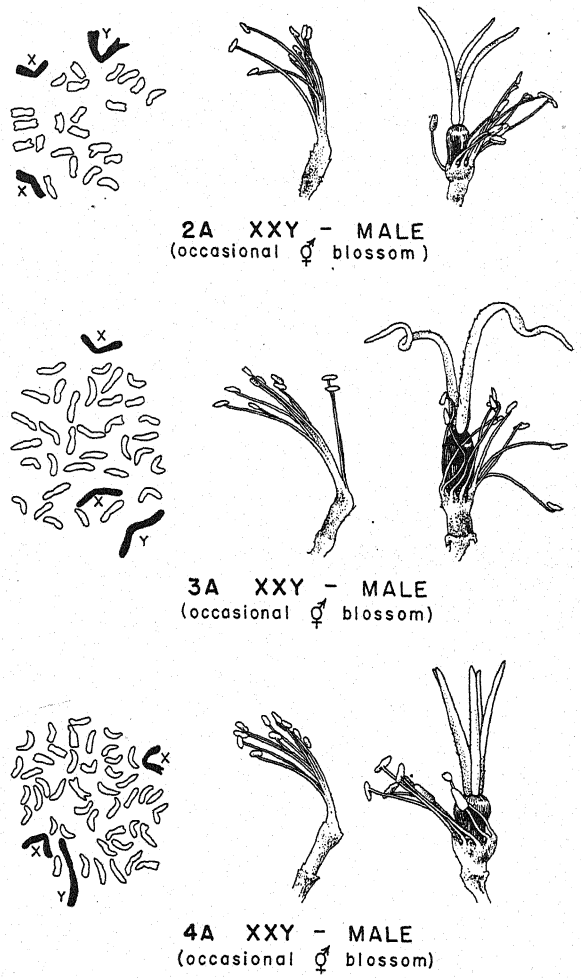


Fig. 6. The autosomes have no primary role in sex determination. As the number of sets of autosomes is increased (2A, 3A, 4A), with the sex chromosome constitution remaining constant, no change in sex expression is observed.

TABLE 3. *Relation of X chromosome-Y chromosome (X/Y) ratio to sex in Melandrium.*

Chromosome constitution	X/Y ratio	Sex
2A XYY	0.5	Male
2A XY 3A XY 4A XY 4A XXYY	1.0	Male
4A XXXYY	1.5	Male
2A XXY 3A XXY 4A XXY 4A XXXXYY	2.0	Male (occasional ♂ blossom)
3A XXXY 4A XXXY	3.0	Male (occasional ♂ blossom)
4A XXXXY	4.0	Hermaphrodite (occasional ♂ blossom)

blossoms, and these were XXY and XXXY types, where an occasional hermaphroditic blossom is expected. All the others were either male or female and in strict accordance with the sex-chromosome constitution: XY, XXY, and XXXY being male, and XX, XXX, and XXXX female. Westergaard's conclusion that male- and female-determining genes are present in certain of the autosomes does not seem to be borne out in these pedigrees.

Further evidence of inactivity of the autosomes in sex determination is seen in the behavior of 36 primary trisomic females (2A+a XX)[2] obtained from crosses of triploid by diploid. Among these 36 plants, many of the eleven different autosomes are probably present as the extra chromosome—just how many we are unable to say because of inability to distinguish between individual autosomes cytologically. There are five or six recognizably different habit types among the 36 plants, however, and these in all probability represent at least that many different autosomes present as trisomics. If any of these extra autosomes have a special tendency towards maleness, one might expect hermaphroditic tendencies among some of the plants. Yamamoto, as noted above, observed in *Rumex* a definite effect of individual autosomes in primary trisomic plants. All these 36 plants in *Melandrium,* however, were strictly female. Up to the present, only one trisomic male has been obtained; it has not been possible, therefore, to check for female genes among the autosomes by this method.

Thus, evidence derived from 2A, 3A, and 4A plants with XXY sex-chromosome constitution, and from a study of X/A ratios, seems to indicate the relative unimportance of autosomes in balanced sets, in sex determination. Moreover, a study of the sex of various forms that have unbalanced numbers of autosomes, including $2n + 1$ plants and the offspring of triploids, has failed to uncover any sex tendencies, either male or female, in individual autosomes.

Sex balance.—With a knowledge of the respective tendencies of the sex chromosomes in *Melandrium,* and with the further recognition that the X/Y ratio is the critical one, it is possible to turn to a consideration of the relative strengths of the sex-determining genes in the X and Y chromosomes.

Table 3 shows chromosome constitution, X/Y ratio, and sex, of a series of plants arranged in order of increasing X/Y ratio. It is clear that the Y is relatively strong; it completely prevails against one X and almost completely against 2 X's and 3 X's. Balance apparently is achieved with the composition XXXX/Y, at which point the plant is almost equally male and female. Even the XXXXY plants, however, produce an occasional staminate blossom, but never a carpellate blossom. This probably indicates that a complete balance has not been reached and that one Y is slightly stronger than four X's in these stocks.

It is evident, furthermore, that the X/Y balance is a matter of threshold. 2A XYY and 2A XY plants are clearly distinguishable phenotypically because of the smaller diameter of the calyx of the former, but both are strictly male. XXXYY plants are also strictly male. Though they possess X chromosomes, and therefore genes for femaleness, these all are below the threshold for expression in the form of female structures. X/Y ratios of 2.0 and 3.0 are sufficiently high for femaleness to show itself, apparently, only when both internal and external environmental conditions are favorable. Less than 10 per cent of the blossoms on these plants have recognizable female structures, and probably less than 1 per cent have female structures sufficiently well developed to produce ovules capable of fertilization.

The next shift in X/Y ratio, from 3.0 to 4.0 however, is crucial, and causes a much greater visible increase in femaleness than all the previous steps in the series combined. Nearly all XXXXY blossoms have well-developed pistils, which set abundant seed. This is interpreted as indicating a typical reaction curve, in which little femaleness is manifest until a critical threshold has been passed and then the addition of a single X is able to cause an almost complete transformation to hermaphroditism.

It seems likely that increase in X/Y ratio beyond 4.0 might cause a further increase in female development, because the pistils of XXXXY plants are not so well developed as those of XXXX plants; but whether a point might be reached where maleness would tend to be suppressed by excessive femaleness (as femaleness is by an excess of maleness) is not known. An effort has been made to obtain XXXXXY plants by intercrossing various tetraploid males and females having extra sex chromosomes, but such efforts to date have not been successful.

[2] Here and elsewhere "a" is used to indicate a single autosome; while "A" indicates a full set of 11 autosomes.

DISCUSSION.—*Sex potentiality vs. sex determination.*—In *Drosophila* and other X/A balance types, all males and females have male and female determiners. Both sexes have male determiners in the autosomes; they differ only in having one or two X's—that is, in the number of female determiners. In *Melandrium,* however, the location of the principal male determiners in the Y, and of the female determiners in the X gives rise to a different condition. The male, having both X and Y chromosomes, has determiners for both sexes, but the female, having X's only has only female-determining factors. Does this mean that XX individuals are completely female in *Melandrium,* without any trace of maleness? Obviously not, for very young blossoms of male and female plants are indistinguishable; both have stamen as well as pistil primordia and are thus fundamentally bisexual. Moreover, it has been known for a long time (Strassburger, 1900) that female plants, when infected with the fungus *Ustilago violacea,* produce mature blossoms with well developed stamens (filled with fungous spores) as well as fertile pistils.

This apparent contradiction may be resolved if one draws a formal distinction between sex "potentialities" and sex "determiners." Females in *Melandrium* have sex potentialities (both male and female), but they have only female determiners. Correns (1928) has designated the potentialities for the formation of female structures as G, and those for the formation of male structures as A. Essentially these are basic reaction systems, necessary for the development of all sex structures, whether male or female, and apparently are present in a homozygous state. This latter condition would seem to locate them chiefly in the autosomes.

Sex-determining factors—or "relizators" (Correns, 1928), "sex-deciding" factors (Muller, 1932a), "sex tendency" factors (Allen, 1932)—are those which act in conjunction with sex potentialities to cause either male or female structures to develop. Sex determiners may well complete an essential step in the complex series of events in sex development, and possibly they are distinguished from sex potentialities only by reason of their position, which is such that their proportion is altered by the sex-chromosome mechanism. As Muller has said, they constitute the trigger which sets off development in one direction or the other.

It is therefore not surprising that female plants should have certain male characteristics and be able to express them in varying degrees under different conditions—as, for example, when infected by *Ustilago*—even though they have no demonstrable male sex determiners. It is not inconceivable in this case that the fungus may act as a partial substitute for the genes of the Y chromosome in producing stimulation or substances that function as male sex determiners.

Role of the autosomes.—There is general agreement among all those who have studied the distribution of the sex genes in *Melandrium* that the Y chromosome is strongly male-determining. There is some disagreement, however, as to the importance of the autosomes in sex determination. Westergaard (1940) has concluded on the basis of two aneuploid intersexes (3A XXY-2a and 4A XXXY-a) that some autosomes must contain male-determining genes. Löve (1944) apparently agrees with this conclusion, although experimental evidence is not presented. It seems possible, however, that the two aneuploid hermaphrodites reported by Westergaard may have resulted from inherent X/Y unbalanced, similar to that observed by Warmke and Blakeslee and by Löve in euploid XXY and XXXY types, rather than from the autosomal deficiencies.

Westergaard also believes that ". . . . female determining genes are found in the X chromosome as well as in the autosomes," and, because of his discovery of an XXXXY plant which was male, that ". . . . it is improbable that the X chromosome may contain female determining genes of any importance." His evidence for female-determining genes in the autosomes, however, comes from what appears to be an error in interpreting data previously published from this laboratory. To quote from Westergaard, page 89: "It appears, too, from Blakeslee's and Warmke's preliminary report (1939b) [Warmke and Blakeslee, 1939b] that female determining genes are to be found in the X-chromosome as well as in the autosomes . . . . because they find a hypotetraploid XXXY-1 plant which is δ . . . . This suggests that female determining genes are found in the autosomes."

We did not report a "hypotetraploid XXXY-1 plant" to be hermaphroditic, and unless there is some error in translation it is difficult to understand how this could have been interpreted as indicating autosomal female genes, in any event. There is a strong tendency for balanced 4A XXXY plants to be hermaphroditic in our stocks (Warmke and Blakeslee, 1939b, 1940c), and a single aneuploid hermaphrodite could not constitute evidence for or against autosomal sex determiners. Moreover, if hermaphroditism had been caused by an autosomal deficiency in this case—this would be evidence for the presence of male-determining rather than female-determining genes in the lost autosome.

Strength of male genes in the Y chromosome.—It is now clear that the strict separation of sexes, for which *Melandrium* is noted, is due in a large measure to the exceptional strength of the male determiners in the Y chromosome. Normal 2A XX individuals have no Y, and therefore no male determiners of importance, and are strictly female.[3]

[3] The report of Åkerlund (1927) of a male-sterile hermaphrodite with female chromosome constitution appears to be explainable on the basis of recent findings of this laboratory. Certain male-sterile hermaphrodites have been found with two X chromosomes and a very small Y fragment. Sometimes this fragment is lost from portions of the sporogenous tissue during development and may give pollen mother cells from entire anthers in which only the two X chromosomes remain.

In the normal 2A XY individual, the male-determiners of the Y chromosome are sufficiently strong to suppress the female influence of a single X chromosome, so that maleness results. As was seen above, one Y must be opposed by three or four X's before appreciable hermaphroditism results. Mutation or selection that considerably increases the strength of female determiners in the X or considerably decreases the strength of male determiners in the Y must occur therefore, before regular hermaphroditism appears in *Melandrium*.

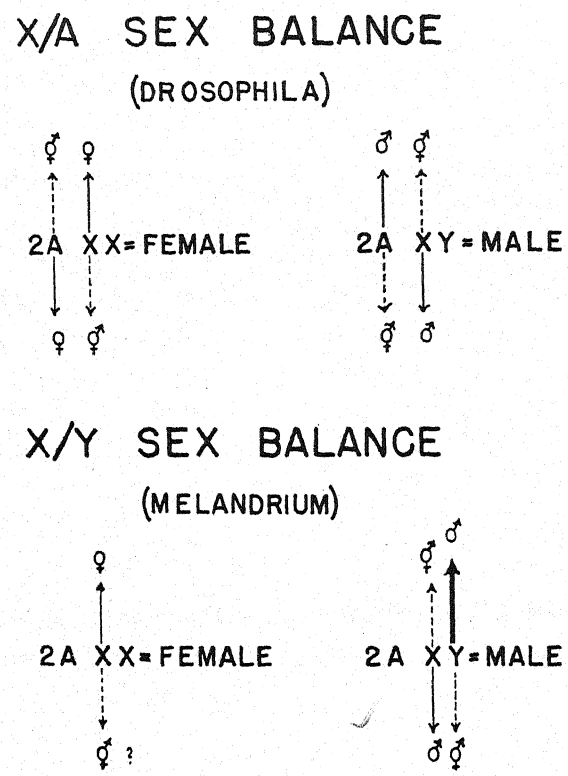


Fig. 7. Diagram illustrating the effect on sex expression of theoretical genetic increases (arrows pointing upward) and theoretical genetic decrease (arrows pointing downward) in strength of sex determiners. All determiners are held within limits by the threat of hermaphroditism (or intersexuality)—except those in the Y chromosome in forms with X/Y sex balance, where there is no limit to the increase in strength of male determiners. It is suggested that this may explain the unusual strength of the male genes in the Y chromosome of *Melandrium*.

It is the exceptional strength of the male-determiners in *Melandrium*, causing 4A XXXY individuals to be male or essentially so, that has made possible the establishment of a 4*n* dioecious race.[4] This exceptional strength of male-determiners, making XXY and XXXY individuals male, was not expected on the basis of previous sex-balance studies (*Drosophila, Rumex*). In these forms any significant change in relative strengths of sex genes will lead to hermaphroditism or intersexuality. This is shown diagramatically in figure 7, top. In this figure arrows pointing up or down indicate possible increases or decreases, respectively, in strength of sex genes. Solid lines indicate changes that lead to intensification of already existing maleness or femaleness; broken lines indicate changes that result in hermaphroditism. It is clear that a sufficient increase (in the male) or decrease (in the female) in the strength of female-determining genes in the X chromosomes must lead to hermaphroditism. It is also true that a sufficient increase (in the female) or decrease (in the male) of the strength of the male-determining genes in the autosomes must result in hermaphroditism. And hermaphroditism in species where there has been a previous evolution of separate sexes is likely to have decreased survival value, not only because of the resulting excessive variability, but also because of the possibility of intersexuality and sterility (*Drosophila*) and self-fertilization and inbreeding (higher plants). The relative strengths of the sex genes in species with X/A balance, therefore, are maintained within narrow limits, neither male nor female genes being allowed to increase or decrease in strength far beyond the other.

In species with X/Y sex balance, of which *Melandrium* is an example, there are upper limits (in the male) and probably lower limits (in the female) to change in strength of female genes in the X chromosome, beyond which hermaphroditism or sterility must result (fig. 7, bottom). There is also a lower limit to change in strength of male genes in the Y chromosome (in the male), *but no upper limit!*

The strength of male-determiners in the Y chromosome of *Melandrium* thus is not limited by hermaphroditism and sexual instability, as are the male genes in X/A species. On the contrary, the greater the divergence in strength of male and female determiners in XY individuals of *Melandrium*, the more sexually stable such individuals will be. This may well have favored increase in strength of male determiners in the Y and decrease in strength of female determiners in the X chromosomes in the manner pointed out by Muller (1932b), by providing ". . . . a margin of stability and security to insure the organism against weakening or excessive variability of the character by other and more common influences—environic and probably also genetic."

Moreover, there does not appear to be the opposing tendency—present in types with autosomal male determiners—for female genes in the X to be maintained at a certain level of potency in order to preserve constancy of sex expression in XX individuals. The female determiners, being unopposed by male determiners in females, have been stabilized at a level just sufficient to evoke the formation

[4] Recent studies on *Acnida tamariscina* (Murray, 1940) and on *Silene otites* (Warmke, 1942), in which 4*n* dioecious races—presumably consisting of XXXX females and XXXY males—also have been produced, may indicate strong male sex determiners in these species too.

of female sex structures. There may well be a direct relationship, therefore, between the unusual strength of the male determiners and the relative weakness of the female determiners in *Melandrium*, as contrasted with *Drosophila* and other X/A forms, and the fact that *Melandrium* has Y-borne male genes and X/Y sex balance.

SUMMARY

Numerous polyploid and aneuploid plants of *Melandrium dioicum* have been obtained and used to study the role of the X chromosome, of the Y chromosome, and of the autosomes in sex determination. The following series: (1) 2A XX, 2A XXY, (2) 4A XXX, 4A XXXY, (3) 4A XXXX, 4A XXXXY, and (4) 4A XX, 4A XXY, 4A XXYY, in each of which the number of Y chromosomes is the sole variable, indicate that the Y has strong male-determining genes.

The series (1) 2A XY, 2A XXY, and (2) 4A XY, 4A XXY, 4A XXXY, and 4A XXXXY, in which the number of X chromosomes is the variable, indicate that the X contains female determiners, but less potent than the male determiners of a single Y.

Evidence derived from 2A XXY, 3A XXY, 4A XXY plants and from an investigation of the X/A ratio, which may range from 0.5 to 1.5 without altering sex, indicate a relative unimportance of the autosomes in balanced sets in sex determination. Moreover, a study of a number of trisomic plants and of the offspring of triploids has failed to show any sex determiners, either male or female, in individual autosomes.

A brief review of the literature relating to the location of sex determiners is given, including a discussion of methods and some theoretical considerations.

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STUDIES IN THE GENUS SCIRPUS L. VIII. NOTES ON ITS TAXONOMY, PHYLOGENY, AND DISTRIBUTION [1]

Alan A. Beetle

PREVIOUS PAPERS of this series (Beetle, 1940–1944) have attempted to treat the species of *Scirpus* (Cyperaceae) in as nearly natural sections as possible. The large size of the genus (about 120 species) and the scattered, world-wide distributions have multiplied the difficulty of presenting immediately a finished treatment. Material of all but the American and European species has usually been difficult to obtain. The gradual accumulation of information through search in herbaria, fortuitous stumbling on the scattered and often unindexed publications on subspecific categories, correspondence, and exchange has resulted in the following notes. The notes are aimed at a clarification of both the genus and its sections through an improved understanding of the species. The species are the units of the evolutionary trends in the genus and to discover the pattern (phylogenetic or distributional) underlying these trends has been the aim of all these studies.

In 1940 (Beetle, 1940–1944) when the subgenera Euscirpus and Aphylloides were proposed as more natural than the old Euscirpus and Isolepis it was agreed that Monoyer (1934) was correct in his choice of a leafy species as representative of a primitive condition in the genus. It was also suggested that since Monoyer's treatment was based solely on European material a considerable amplification and modification of his abbreviated phylogenetic tree would be necessary even though his general premise was correct. Monoyer chose *Scirpus sylvaticus* and *S. maritimus* and their allies (his section Phylloscirpus) as most representative of the primitive condition at least as far as the vascular system is concerned. However there are considerable differences between *S. sylvaticus* (Section Nemocharis) and *S. maritimus* (Section Reigera) (cf. Beetle, 1940–1944) and there are a good many other equally leafy species whose anatomy Monoyer did not study. The need is then still apparent to select a species as nearly representatively primitive as is possible, bearing in mind that all species may be equally primitive in some of their characters and that no species is wholly primitive in all its characters.

This species should combine (1) leafiness (characteristic of Euscirpus but not of Aphylloides) with (2) an open paniculate inflorescence (common throughout Euscirpus but often much reduced in Aphylloides), (3) an achene roughly 2 mm. long (a size common throughout Aphylloides but rarer in Euscirpus where the achene is usually 1 mm. long, *cf.* Beetle, 1943), (4) a perennial habit (the annuals appear in only two sections, Isolepis and Actaeogeton of Aphylloides), (5) many-flowered spikelets of intermediate size (relatively very few reduced and otherwise specialized species in the genus are few-flowered, and also small-spikeleted; extra large spikelets are also unusual; it would be difficult without much supporting evidence to imagine either the few species with small, few-flowered spikelets, *e.g.*, Section Baeothryon, or those with large spikelets, *e.g.*, Section Reigera, evolving the many other types present in the genus), (6) a perianth of six setae about equaling the achene (this is the most common condition in both subgenera).

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1. Section **Hymenochaeta** (Beauv.) comb. nov. *Hymenochaeta* Beauv. in Lestib. Ess. Fam. Cyp. 43. 1819, as genus, type species *Scirpus grossus* L.f.; Nees & Mey. Linnaea 9: 293. 1934.

In the Section Hymenochaeta the conditions set forth above are all met, especially as the section is represented by the following: *Scirpus grossus* L. f. Suppl. 104. 1781.

Isolepis grossa Wall. Cat. n. 3470; *Hymenochaeta grossa* Nees, Edinb. N. Jour. 264. 1834; *Schoenoplectus grossus* Palla. Allg. Bot. Zeitschr. 17: Beil 3, 1911. *Scirpus aemulans* Steud., Zoll. Syst. Verz. Ind. Arch. 2: 62. 1854; *S. maritimus* var. *aemulans* Miq. Fl. Ind. Bot. 3: 306. 1859. *S. maximus* Roxb. Fl. Ind. 1:132. 1820; *Hymenochaeta maxima* Nees, Linnaea 9: 293. 1834; *Isolepis maxima* A. Dietr. Sp. Pl. 2: 130. 1833. *Scirpus griffithii* and *S. scaberrimus* Boeck. Flora 41: 595. 1858. *Hymenochaeta haemotodes* Nees & Mey. Linnaea 9: 293. 1834. *Isolepis giganteus* Roxb. in Wight. Contrib. Bot. Ind. 110. 1834.

Perennial; rhizomes slender, ca. 5 mm. thick, culms 1 to several from a thickened corm-like base, up to 2 m. tall, the smooth, triangular stems soft and spongy, the sides up to 2.5 cm. broad, the leaves basal, as broad as the culms, smooth; involucral bracts leaflike, usually three, the outer often 5 dm. long, the others shorter, far exceeding the panicle; panicle usually not over 2 dm. long, with primary, secondary and tertiary rays, the rays terete and minutely scabrous; scales reddish brown; bristles six; style trifid; achene trigonous. (Type locality, India.)

A variation from India has the glumes more mucronate and the bristles villous but the characters are not of a degree which has served for the separation of either species or varieties in other sections or in other floras, and should be regarded as **Scirpus grossus** L. f. **forma kysoor** (Roxb.) comb. nov. *S. kysoor* Roxb. Fl. Ind. 1: 235. 1820; *S. grossus* var. *kysoor* (Roxb.) Clarke in Hooker, Fl. Brit. Ind. 6: 659. 1894.

Scirpus grossus L. f. is easily separated from the only other member of the section, *S. ternatensis* Reinw., for the former has its spikelets single on the rays, the latter in groups of three to many.

Having settled on this species as representative of a primitive condition in the genus the relative primitiveness of a number of other contrasting characters becomes more apparent, some of these changes appearing independently many times (*e.g.*, bristles absent as contrasted with bristles present). Thus a more primitive condition is indicated by the trifid style, the trigonous achene, the trigonous culm, and scabrous parts as compared with the derived bifid style, lenticular achene, round culm, and smooth parts.

These conclusions also lead to the suggestion that since the distributions of the species of the section Hymenochaeta are Asiatic, Asia is the section of the world where the genus originated and from there the tracing of distribution patterns should begin. This area has long been recognized as the cradle of other now wide-spread genera.

2. Section Androcoma Nees.

Since the species of this wholly American section are more nearly similar to those of Section Hymenochaeta than to any other American species this section is taken to be the most representatively primitive of those sections that are represented in the Americas. The section may be divided into two well-marked series (Beetle, 1940–1944). A treatment of one of these, Series Lineatae, is finished (Beetle, in press). A new species and a new variety, both from South America, here are added to the other, Series Euandrocoma.

The following variety is a recently collected, strongly contrasting variation of the common South American plant *Scirpus subasper* Beetle (see Univ. of Cuzco, Rev. Universitaria, 1945; *S. glaucus* Nees, Linnaea 9: 293. 1834, not Torrey, 1924, nor Lam., nor Smith; as illustrated by Barros, Ann. Mus. Arg. Cienc. Nat. 38: 166. fig. 15. 1935).

Scirpus subasper Beetle var. **diffusus** Beetle, var. nov.

Similis *Scirpo subaspero* Beetle a quo spiculis solitariis in inflorescentiae ramis rachis longioribus (usque ad 7 cm. longis) differt.

The spikelets in the typical and widespread *S. subasper* are in glomerules of three to eight spikelets. In var. *diffusus* Beetle the spikelets are single on elongate rays.

Type locality: Chile, Prov. Coquimbo, Dept. Illapel, Caren, *Worth & Morrison 16491.* (Type in the author's herbarium.)

Scirpus **angustisquamis** Beetle, sp. nov.

Culmi laeves, valde triangulares, faciebus usque ad 6 mm. latis; involucri bracteis 3-multis, usque ad 3.5 dm. longis, inaequalibus, margine scabris; spiculis saepe viviparis; radiis primariis usque ad 1 dm. longis, laevibus; spiculis 3-multis, glomeratis; squamis usque ad 3 mm. longis, angustis, marginibus circum achaenium involutis; setis absentibus; stylo trifido; achaenio 1.5 mm. longo, griseobrunneo, lenticularis.

Culms smooth, strongly triangular, the sides up to 6 mm. broad; involucral bracts 3 to many, up to 3.5 dm. long, strongly scabrous, unequal; inflorescence sometimes viviparous; primary rays up to 1 dm. long, flattened, smooth, secondaries up to 3 cm. long, terete, smooth; spikelets 3—many in glomerules; the noticeably pedicelate florets somewhat distichous; scales up to 3 mm. long, narrow, the margins inrolled about the achene; bristles absent; style trifid; achene 1.5 mm. long, slender, gray-brown, lenticular.

Type locality: Chile, Prov. Valdivia, Estacion Mailef, Cassila de Borreos 31; San Jose de la Mariquine, Coll. *Pablo Aravena* no. 7. (Type in the author's herbarium.) Only other collection seen: Chile: Angol, Nov. 21, 1933, *R. Marillan 180* (Herb. Univ. of Calif., Berkeley).

3. Section NEMOCHARIS Beurl.

Study of a recent loan of material from the New York Botanical Garden has resulted in the identification of two new Asiatic species in this section:

SCIRPUS **helferi** sp. nov.

Perennis; *Scirpo congdoni* simillimus; vaginis subrubris; laminis culmi medium subaequantibus et usque ad 2 dm. longis; culmis triangularibus (ut pro *S. chilensi*), laevibus involucri bracteis 3–5; spiculis 2–5 radio quoque dispositis; squamis subrubris, multinervatis, apice mucronatis; stylo trifido; achaenio trigono, 1.5 mm. longo, laevi.

Perennial, in general appearance very suggestive of *Scirpus congdoni* of the North American Sierra Nevada; leaves few and mostly basal, the sheaths reddish, the blades up to 8 mm. broad, smooth, up to 2 dm. long; culm sharply winged-triangular (in the same manner as *S. chilensis*), smooth, involucral bracts 3–5, unequal, leaflike except for the scabrous margins, rays terete or angular, smooth; spikelets 2–5 on the rays, 2–5 mm. long; scales reddish, very broad at base, the tip slightly mucronate, prominently nerved; style trifid; achene smooth, yellow, markedly winged trigonous, 1.5 mm. long.

Type locality: India, Tenasserim and Andamans, Herb. Helfer, Herbarium of the late East India Company no. 6164, distributed at the Royal Gardens, Kew. 1862–3. (Type in the Herbarium of the New York Botanical Garden.)

SCIRPUS **hokkaidoensis** sp. nov.

Perennis; culmis usque ad 5 dm. altis, apice acriter triangularibus, laevibus, basi vaginatis; vaginis non rubidis, lamina summae vaginae culmum aequante et usque ad 1 cm. lata; involucri bracteis quam umbella brevioribus; radiis primariis usque ad 1 dm. longis; spiculis in inflorescentiae ramis unice dispositis; bracteis 1.75 mm. longis, ovato-lanceolatis, glabris; setis hypogynis 6. glabris; stylo trifido; achaenio compresso-trigono, 1.25 mm. longo, apiculato.

Perennial; culms stout, sharply trigonous above, obscurely below, the angles smooth, up to 5 dm. tall, very leafy, the sheaths smooth, not reddish, the blades up to 1 cm. broad, only the upper equaling or exceeding the culm, scabrous on the margins; involucral bracts leaf-like, shorter than the ample compound umbel; primary rays up to 1 dm. long, the spikelets single on the secondary and tertiary rays; rays terete, smooth; scales 1.75 mm. long, ovate-lanceolate, glabrous; bristles 6, several times longer than achene but included, glabrous; style trifid; achene compressed-trigonous, 1.25 mm. long, apiculate.

Type locality: Japan, Hokkaido, Meakan, Aug. 2, 1929, *T. tanaka 168*. (Type in the Herbarium of the New York Botanical Garden.)

4. Section TRICHOPHORUM Pers.

This section consists of four species and two varieties, all of eastern North America except for one species from Asia which although well represented in herbaria has never been described. The entities may be keyed as follows:

All spikelets in glomerules of three or more
  Inflorescence not capitate
    1. *S. cyperinus* (L.) Kunth, Enum. Pl. 2: 170. 1837.
  Inflorescence capitate
    2. *S. cyperinus* f. *cephaloideus* (Sheldon) Beetle, Rhodora 46: 146. 1944.
At least the lateral spikelets of each group pediceled
  Involucels and wool brown or reddish
    Lateral pedicellate spikelets mostly double; achene 1.25 mm. long
      3. *S. asiaticus* Beetle (see below)
    Lateral pedicellate spikelets single; achene 1 mm. long
      Involucels and wool bright red-brown or terra-cotta
        Inflorescence not capitate
          4. *S. cyperinus* var. *eriophorum* (Michx.) Kuntze, Rev. Gen. Pl. 2: 757. 1891.
        Inflorescence capitate
          5. *S. cyperinus* var. *eriophorum* f. *confertus* Beetle (see below).
      Involucels and wool dull brown, not reddish
        6. *S. cyperinus* var. *laxus* (Gray) Wats. & Coult. in A. Gray, Man. ed. 6. 582. 1890.
  Involucels black, the wool nearly white
    Achene white; bristles somewhat exserted
      Inflorescence not capitate
        7. *S. atrocinctus* Fern. Proc. Am. Acad. 34: 502. 1899.
      Inflorescence capitate
        8. *S. atrocinctus* f. *brachypodus* Blake, Rhodora 15: 161. 1913.
    Achene reddish; bristles long exserted
      9. *S. longii* Fern. Rhodora 13: 6. 1911.

SCIRPUS **asiaticus** sp. nov.

Caespitosa; culmis obscure trigonis, usque ad 1.5 m. altis; laminis culmum aequantibus, marginibus valde scabris; involucri bracteis 3–5, usque ad 2 dm. longis, quam umbella brevioribus; radiis primariis usque ad 1.5 dm. longis, scabris; spiculis solitariis vel duabus in ramis, 3–6 mm. longis, ovoideis; bracteis 2 mm. longis; setis hypogynis 6, longe exsertis, laevibus, crispis flexuosisque; stylo trifido; achaenio 1.25 mm. longo, trigono, brunneo, apiculato.

Tufted perennial; culms obscurely trigonous, 1–1.5 m. high; basal and culm leaves numerous, up to 1 cm. broad, often equaling the culm, strongly scabrous on the margins; involucral leaves 3–5 (up to 2 dm. long, usually shorter than the ample inflorescence (up to 3.5 dm. long); primary rays up to 1.5 dm. long, scabrous, involucels reddish brown, secondary rays up to 1 dm. long, scabrous, the tertiaries numerous, spikelets mostly either single or in pairs, 3–6 mm. long, ovoid; scales 2 mm. long, reddish; bristles 6, reddish-brown, elongate; style 3-fid; achene 1.25 mm. long, trigonous, light brown, apiculate.

Type locality: China, Yunnan Prov. *A. Henry 12527* (Type in the Herbarium of the New York Botanical Garden).

All the identified sheets I have seen of this species have been referred either to *S. cyperinus* (L.) Kunth or to *S. eriophorum* Michx. The achenes of these two measure up to 1 mm. long including the apiculate tip. The body of the achenes of *S. asiaticus* consistently measure 1 mm., the apiculate tip bringing the total measurement to 1.25 mm. This basic difference (of the same order of magnitude as the difference in the achenes of *S. microcarpus* Presl and *S. rubrotinctus* Fern.) coupled with the dull reddish-brown color of the scales and bristles (*S. cyperinus* is grayish and *S. cyperinus* var. *eriophorum* is a bright red-brown) and the distinctive grouping of the spikelets suggests that the Asiatic material is specifically different from anything found in North America.

Specimens examined include: Japan: Prov. Ugo, Mt. Kuromori, *K. Yushun* in 1905. China: Prov. Kiangsi, *Stewart & Cleo 371, Tsiang 10236 & 10729,* and *Chung & Sun 713;* Prov. Yunnan, *Henry 12527, Maire 6807 & 3058,* and *Wilson 1632;* Prov. Anhwei, *Sun 1339;* Prov. Shantung, *Chiao 2817.* Korea, *Smith.* India, East Bengal, Herb. *Griffith 6266;* Shillong, *Clarke 38756;* Sohra, *Clarke 17480;* Assam, *Kurz* Herb.

SCIRPUS CYPERINUS (L.) Kunth var. ERIOPHORUM (Michx.) Kuntze forma **confertus** Beetle, f. nov.

Scirpo cyperino var. *eriophoro* similis sed a typo differt spiculis in inflorescentiae ramis congestis.

As in *Scirpus cyperinus* var. *eriophorum* but spikelets congested on short rays into crowded heads.

Type locality: New York, Long Island, Millneck, Aug. 30, 1921, *W. C. Ferguson 800.* (Type in the Herbarium of the New York Botanical Garden.) Other specimens seen: from type locality, *Ferguson 801;* from Long Island, Hempstead, *Ferguson 647.*

Often referred to this section is the following:

Eriophorum maximowiczii (Clarke) Beetle, comb. nov.

Scirpus maximowiczii Clarke, Kew Bull. ser. 8. 30. 1908.

Clarke, himself, suggested "ad genus *Eriophorum* potius referenda" and that this certainly is the case was decided after a review of the sections of *Scirpus* into which the species might fall. The achenes of the species are more like those of species of *Eriophorum* than those of the section Trichophorum (Beetle, 1943), the most satisfactory section for it in *Scirpus.* The same arguments presented (Beetle, 1942) in the transfer of *Scirpus criniger* Gray to *Eriophorum* may be reapplied here with the same result: The species is known only from Japan.

5. Section Schoenoplectus Palla.

This section has been treated as a whole by Beetle (1940–1944). Since that time a detailed comparison of the South and North American material of *Scirpus nevadensis* indicated that the Patagonian plant is uniformly smaller with short, spreading leaves, a sharp contrast to the taller plant of western North America which is characterized by its stiffly erect leaves which about equal the culm. The following combination is made with these differences in mind in order that the South American plant may be treated as a variety of the North American:

Scirpus nevadensis Wats. var. **remireoides** (Griseb.) comb. nov. *Scirpus remireoides* Griseb. Pl. Lorent. 218. 1874.

The North American material of *Scirpus chilensis* Nees & Meyen (commonly known as *Scirpus olneyi* Gray) differs from the South American type in its uniformly 2-fid style and stouter culms with markedly winged angles and therefore should be treated as a variety of the South American species:

Scirpus chilensis Nees & Meyen var. **longisetis** (Kük.) comb. nov.

Scirpus olneyi var. *longisetis* Kük. Arkiv. Bot. 22A, no. 17: 8. 1929.

These two combinations bring to mind the considerable similarities between the *Scirpus* floras of the temperate regions of the Americas (Beetle, 1941). The following list indicates a distribution pattern and catalogues the strength of the differences between the contrasting elements.

Temperate North America	Temperate South America
S. analecti	*S. subasper*
S. clementis	*S. atacamensis*
S. nevadensis	*S. nevadensis* var. *remireoides*
S. chilensis var. *longisetis*	*S. chilensis*
S. americanus	*S. americanus* var. *longisetis*
S. americanus var. *polyphyllus*	*S. americanus* var. *polyphyllus*
S. californicus	*S. californicus*
S. cernuus var. *californicus*	*S. cernuus* var. *pygmaeus*

It is distributionally interesting to contrast the entities in this list with the comparative elements of the European and North American temperate zones wherein may be seen the outlines of a northern hemisphere transatlantic distribution pattern in *Scirpus:*

Europe	Eastern North America
S. hudsonianus	*S. hudsonianus*
S. pumilus	*S. pumilus*
S. cespitosus	*S. cespitosus* var. *callosus*
S. maritimus	*S. maritimus* var. *agonus*
S. supinus	*S. supinus* var. *hallii*
S. sylvaticus	*S. sylvaticus* var. *bissellii*
S. americanus var. *triangularis*	*S. americanus*
S. lacustris	*S. validus*

On the other hand the Pacific Northwest route through Alaska to Asia offers no such contrasting elements in the floras and offers relatively little to a study of migration patterns in *Scirpus.*

6. Section Paucispicata Beetle.

The new sectional name Paucispicata is proposed for a little collected and small group of closely related Asiatic species. As treated here it comprises four species and a variety. All the collections are from southeastern Asia and nearby islands.

The section Paucispicata, having only rudimentary leaf-blades and non-leafy, inconspicuous involucral bracts is included in the subgenus Aphylloides. Previously suggested relationships for the species of this section include Blysmus by Clarke (1908, Kew Bull. Add. Ser. 8. 111–113) but the other species there have been separated from *Scirpus* by Beetle (1940–1944) and previous authors on the basis of the spicate inflorescence and additional characters which are not found in the species treated here. Merrill (1910) when proposing *S. pulogensis* suggested a relationship to *S. pauciflorus* which is now treated as *Eleocharis*. A study of the single spikelet of *S. clarkii* (*S. pulogensis*—see below) shows that it is not terminal in the same sense as that found in *Eleocharis* nor do the achenes of any of the species of this section have the tubercle which characterizes the achenes of *Eleocharis*.

Since the section Paucispicata approaches the section Baeothryon in many vegetative characters and in the similarity of the achenes, it is necessary to emphasize the differences. For most of the entities treated here the multiplication of spikelets is at once recognized as not found in section Baeothryon although at first glance this seems not to hold for *Scirpus clarkii*. However, in this species a close examination of the single spikelet reveals that it is not truly terminal in the same sense as are spikelets found in section Baeothryon and in *Eleocharis*. In *S. clarkii* the outer scale completely sheathes the apex of the culm and hides a short but evident pedicel which bears the spikelet. In Asia the species of the section Baeothryon are in the Himalaya Mountains or north and westward, at no point overlapping the range of the species of Paucispicata.

The material available has consisted of 46 sheets representing 30 different collections from 21 localities. Additional collections reported in the literature but not seen include:

(1) *S. subcapitatus* according to A. Trimen in Handbook of the Flora of Ceylon 5: 77. 1900: "Also in Nilgiris, Sumatra, and China."

(2) *S. clarkii* collected by Ridley on Mt. Kinabalu as reported by Ridley in Flora of the Malay Peninsula.

(3) *S. subcapitatus* from the Deccan Peninsula as reported by Stapf when describing *S. pakapakensis*.

(4) the type collections of *S. clarkii* and *S. pakapakensis* from British North Borneo.

(5) a sheet from Sumatra, *Beccari no. 276* on Mt. Singalan as reported by Stapf when describing *S. clarkii*.

(6) *S. mattfeldianus* from the Lower Yang-tse-Kiang, collected by *Maries* as reported by Stapf when describing *S. clarkii*.

Only six names, one of them a *nomen nudum*, are involved in the treatment of the entities. Although in no case have the actual types been seen, three type collections were found and a topotype. All the names suggested for this group have been described under *Scirpus*.

The wiry stems of *S. mattfeldianus*, which is abundant in the few localities where it occurs, are used for making ropes and mats.

In the study of this section material in the herbaria of the following institutions has been consulted: Gray Herbarium, Harvard University (G); University of California (UC); United States National Herbarium (US); New York Botanical Garden (NY); Catholic University of America (CU). There is little, if any, additional material in other herbaria in the United States.

Section Paucispicata Beetle, sect. nov. Herbae perennes, dense caespitosae; rhizomatibus brevibus, lignosis; culmis erectis, basi vaginatis; spiculis unicis vel paucis; stylo trifido; achaenio laevi. Densely cespitose perennials; culms erect from a short, woody rhizome and clothed at the base with persistent sheaths; spikelets one to few; style trifid; achene smooth. Type species, *Scirpus subcapitatus* Thw.

Key to the Species

Inflorescence subtended only by the mucronate sheathing outer scale of the spikelets, the mucro 1–3 mm. long
- Culms terete, smooth
  - Spikelets single on culms; mucros of sheaths and of outer scale scabrous — 1. *S. clarkii*.
  - Spikelets mostly 2–4; mucros of sheaths and of outer scale smooth — 3. *S. subcapitatus*.
- Culms trigonous (sometimes obscurely), scabrous on at least one angle (often obscurely)
  - Scales 4 mm. long, lanceolate-acute; spikelets 1–3, rayed — 2. *S. clarkii* var. *pakapakensis*.
  - Scales 3–3.5 mm. long, ovate; the first spikelet sessile, the other 2–8 pedicellate — 4. *S. mattfeldianus*.

Inflorescence subtended by an outer non-sheathing bract 1–2 cm. long; culms strongly scabrous their entire length — 5. *S. scabriculmis*.

1. Scirpus clarkii Stapf, Trans. Linn. Soc. Ser. II. 4: 244. 1894. *Scirpus pulogensis* Merr. Phil. Jour. Sci. Bot. 5: 333. 1910.

Culms 1.5–6 dm. tall, green, smooth, terete, at least the younger prominently grooved, clothed at the base with brown sheaths bearing mucronate leaf-blades 2–8 mm. long, scabrous on the margins, the orifice 1–2 mm. long, abruptly contracted, with a wide hyaline border; spikelets 8 mm. long, 2 mm. wide, solitary, terminal, 7–8-flowered, lanceolate; outer bract ca. 3 mm. long, completely sheathing the culm at its base, the midrib mucronate, the tip

at most 1.5 mm., usually shorter with scabrous margins; scales 4 mm. long, brown, the outermost obtuse, the inner becoming acuminate; bristles 6, fragile, brown, terete, smooth, exceeding or equaling the achene or abortive; stamens 3; style 3.5 mm. long, the segments ascending-hairy; achene 2–2.5 mm. long, 0.75–1 mm. broad, lanceolate, brown, smooth, trigonous, compressed, the summit acutely apiculate.

Type locality: Mount Kinabalu, British North Borneo (11,000 ft.); *Haviland 1398* (not seen).

Distribution: Moist hollows at high elevations. British North Borneo, Mount Kinabalu, 5–11,000 ft. elevation: "Paka Cave to Low's Peak" *D. L. Topping 1699* (US) (marked as a topotype of *S. clarkii*); *M. S. clemens 10609* (UC, G, US); *M. S. Clemens 32329* (UC); *J. & M. S. Clemens 51657* (G); *J. & M. S. Clemens 51229* (G, UC); *J. & M. S. Clemens 27078* (UC); *J. & M. S. Clemens 33842* (UC). Philippines, Luzon, Mt. Pulog: "May, 1909," *Merrill 6550* (US) (type collection of *Scirpus pulogensis*); *Ramos & Edaño* in 1925 (NY, UC); *M. S. Clemens 5016* (UC); *Santos* in 1918 (G, US, UC); *Merrill 7789* (US); *Merrill 6616* (US, NY); *Curran, Merritt & Zschokke* in 1909 (US). Since all the Philippines collections came from a restricted area at the summit of Mt. Pulog (ca. 2,700 ft. elev.) they are topotypes for *S. pulogensis*. Japan, Liu Kiu Islands, *L. Boehmer & Co.*, 1904 (NY).

2. **Scirpus clarkii** Stapf var. **pakapakensis** (Stapf) comb. nov. *Scirpus pakapakensis* Stapf, Jour. Linn. Soc. Bot. 42: 174. 1914.

Culms more wiry than in *S. clarkii*, up to 1 m. tall, obtusely and very obscurely trigonous, obscurely scabrous on one angle; the spikelets single or occasionally two (also three according to the original description) on smooth, terete pedicels. Otherwise identical with *Scirpus clarkii*.

Type locality: Mt. Kinabalu (no. 4277) not seen.

Distribution: moist hollows at high elevations: British North Borneo, Mt. Kinabalu, 8–10,000 ft. elevation: *J. & M. S. Clemens 29002* (UC, G); *M. S. Clemens 10706* (UC). Malay Peninsula, Ganong Tahan, Pahang; elev. ca. 6,000 ft., *R. E. Holttum 20710* (UC).

3. Scirpus subcapitatus Thwaites, Enum. Pl. Zeyl. 351. 1864. *S. subaphyllus* Boeck. Linneae 36: 705. 1870, *nomen nudum* in synonymy of *S. subcapitatus* Thw.

Culms up to 4 dm. tall, green, smooth, terete, grooved, clothed at the base with brown sheaths; the upper with mucronate blades 1–5 mm. long, smooth; outer bract 5 mm. long, sheathing the culm at its base, the mucro smooth; the first spikelet sessile, the other two or three on short (2–3 mm. long) smooth pedicels; spikelets 3–8 mm. long, lanceolate-acute, 7–8-flowered; scales lanceolate-acute, 4–5 mm. long, the broad green midnerve prominently mucronate, the broad hyaline border purple-flecked; style 3.5 mm. long, reddish; stamens 2 mm. long; achene and bristles not seen but according to original description: "Caryopsi obovato-oblonga trigona, laevi; setis 6, tenuibus, denique caryopsi multuplo longioribus."

Type locality: "Ceylon, Hab. Newera Ellia—c.p. 306." Only a sheet of the type collection (G) has been seen.

4. Scirpus mattfeldianus Kük. Fedde Repert. 27: 108. 1909.

Culms up to 1 m. tall, obscurely trigonous and smooth or obscurely scabrous on the angles, the lower sheaths persistent, brown, the upper green, with a mucronate blade up to 2 cm. long, scabrous on the margins; spikelets always several, the first usually sessile the other 2 to 8 spikelets on terete, usually smooth rays up to 2.5 cm. long; outer bract ca. 5 mm. long, sheathing the culm and with a prominently mucronate tip (1–2 mm.) with scabrous margins; spikelets 3–8 mm. long, lanceolate, acute, straw-colored; scales 3–3.5 mm. long, broad at the base, narrowly obtuse or acute above, the midrib sometimes excurrent and scabrous; style reddish; anthers 2.5 mm. long; bristles 6, fragile, elongate, exceeding the achene but not exserted; achene 2 mm. long, brownish-black, smooth, triangular, prominently apiculate.

Type locality: China, Chekiang Prov. based on *Ching 1332* and *1656* (see below).

Distribution: wet places by springs and streams. China: Kwangtung Province, Lung So Tso, Sam Kok Shan, May 18, 1932, *W. T. Tsang 20501* (US, CU, UC, NY); Kwangsi Province, Bin Long, Miu Shan, N. Luchen, border of Kweichow, 4000 ft., June 14, 1928, *R. C. Ching 5960* (NY); Hunan Province, P'ing T'ou Shan, Pai Mu Village, March, 1934, *Tsang 23381* (G, US); Fukien Province, Huong-guong Nang, Temple near Gak-liang, Ing Hok, March 20, 1927, *T. S. Ging 1325* (UC), *H. H. Chung 8474* (NY), and Ing Hok, March 24, 1925, *Chung 3220* (UC); Chekiang Province, Yun Fun, near Taichow, May 1, 1924, *Ching 1332* (G, UC), and near Siachu, May, 1924, *Ching 1656* (UC, US); Anhwei Province, July 15, 1925, Huang Shan (or Wang Shan), *Ching 8621* (UC, US), July 15, 1925, Huang Shan, *Ching 8585* (US, UC), and March 25, 1924, T'ien T'ai Shan, Chu Hwa Shan, *A. N. Steward 5282* (UC).

5. Scirpus **scabriculmis** sp. nov.

Herba perennis, dense caespitosa, rhizomatibus brevibus lignosisque; culmis 2–5 dm. altis, triangularibus, scabris vel ad angulos serrato-denticulatis; vaginis brunneis, apiculatis vel superioribus elongate laminatis, 1–3 cm. longis; involucri ima bractea remota, 0.5–1.5 cm. longa; spiculis 2, pedunculatis (1–3 mm. longis); bracteis 3–4 mm. longis, obtusis, submucronatis; setis 6, achaenio longioribus; stylo trifido, laterculo-rubro.

Culms 2–5 dm. tall, triangular, prominently scabrous their whole length on one angle, the upper sheath bearing an elongate blade 1–3 cm. long, prominently scabrous on the margins; outer invo-

lucral bract 0.5–1.5 cm. long, not sheathing the culm, the margins prominently scabrous, the two spikelets on short (1–3 mm.) scabrous pedicels; spikelets 5–10 mm. long, lanceolate, the outer scale sheathing the pedicel, with midrib excurrent as a prominent scabrous mucro; inner scales 3–4 mm. long, obtuse, the midrib minutely apiculate; style brick-red; bristles six, fragile, elongate but not exserted; achene not seen.

Type locality: Indo-China, Chape, rocks of ravine, Feb. 1931. *A. Pételot 6128* (G).

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EMBRYOGENY AND DEVELOPMENT OF THE APICAL MERISTEMS OF PSEUDOTSUGA. I. FERTILIZATION AND EARLY EMBRYOGENY [1]

George S. Allen

The scope of enquiry of modern morphological studies on the gymnosperms has been restricted to limited phases of the life cycle. From these numerous works has come a rather extensive mass of data dealing with such phases as the development of the proembryo (Buchholz, 1918, 1931, etc.), the early embryogeny of conifers (Buchholz, 1918; Schopf, 1943), the complete embryogeny of *Larix* (Schopf, 1943), the anatomy of the dormant embryo of conifers (Buchholz and Old, 1933; Schopf, 1943; etc.), and the cytohistogenesis of the shoot apex (Korody, 1937; Foster, 1938, 1939, etc.; Cross, 1943; Johnson, 1939, 1944; Kemp, 1943; Sterling, 1945; etc.). No modern work of an intensive nature has dealt with the developmental anatomy of the gymnosperm root apex despite the unsatisfactory state of knowledge which exists (see Guttenberg, 1941).

These numerous investigations have advanced considerably our knowledge of the comparative morphology of the gymnosperms and, in the main, have resulted in a more realistic approach to the problems of growth and development. On the other hand, their limited scope has resulted in failure to produce a connected comprehensive picture of the development of a gymnosperm plant; Schopf's (1943) work on embryogeny most closely approaches this ideal but stops short at the dormant embryo. A truly complete study of the apical meristems of a seed plant should begin with their appearance in embryogeny and continue through successive seedling stages to the apices of large plants. Esau (1943) has pointed out the need for such an investigation dealing with the origin and development of primary vascular tissues.

One of the aims of this study has been to obtain a connected picture of the origin and development of the apical meristems of *Pseudotsuga*, a conifer whose apices may very well represent intermediate stages in the evolution of those of the flowering plants. The goal has not been to add more detail to the already extensive array although this has been inevitably one result.

In a previous paper (Allen, 1943) some details of early embryogeny, fertilization, and sporogenesis in *Pseudotsuga taxifolia* (Lamb.) Britt. were presented. Evidence was given of a polar movement of the free nuclei derived from the zygote. However, a more intensive study of additional material with improved technique has indicated that this interpretation was incorrect—that, in general, the early development of the proembryo is similar to that in *Pinus*. One purpose of this paper is to present statistical evidence in support of the last statement and to include some additional information relative to fertilization and the development of the early embryo. A second paper will deal with later embryogeny and the development of the apical meristems in the larger sporophyte.

Materials and methods.—A comprehensive collection of ovules was made during 1943 in the vicinity of Victoria, British Columbia. A special effort was made to obtain abundant material during the period of fertilization which centered about June 16th. Ovules were slabbed, and killed and fixed in Carnoy's fluid, removed after 24 hours, and stored in 70 per cent alcohol. Material collected on March 29th provided satisfactory stages of meiosis of the megasporocytes.

Material to be sectioned was dehydrated and embedded in paraffin according to Ball's (1941) technique and sections were cut at 14 microns. A con-

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The investigations forming the basis for this series of papers were carried out in the Department of Botany, University of California. It is with great pleasure that the writer acknowledges the encouragement and helpful criticism of Dr. Adriance S. Foster throughout the course of the work.

siderable number of early embryos were dissected from the gametophytes, stained, and mounted whole by Buchholz's (1938) technique.

Various stains were used but the most useful was a modification of Foster's (1934) tannic acid-ferric chloride-safranin combination. This technique resulted in deeply-stained chromosomes and yet rarely stained the so-called "Hofmeister bodies" of the egg; hence the task of counting accurately the number of nuclei within the fertilized egg was rendered relatively simple. For these early stages the usual tannic acid-ferric chloride schedule was followed by two hours in 1 per cent safranin in 50 per cent alcohol with differentiation for a few seconds in a saturated solution of picric acid in 95 per cent alcohol.

PRE-FERTILIZATION DEVELOPMENT.—The mature archegonium consists of a large egg cell, a ventral canal cell which is small and lens-shaped, and a neck composed of a plate of four to eight cells derived from the primary neck cell. Surrounding the mature egg nucleus is a so-called "cytoplasmic sheath" composed of finely granular material (see Chamberlain, 1935, p. 335); this may represent a zone in which is in progress active digestion of the insoluble reserves of the egg. Within the egg cytoplasm are many "Hofmeister bodies" which take up most nuclear stains (fig. 3 and 6).

The egg cell is surrounded by a more or less monolayered "jacket" of cells which are small and deep-staining; these cells remain active throughout the development of the archegonium. The numerous mitotic figures to be seen in the jacket cells immediately after syngamy provide many polar views of chromosomes. It was possible to establish the gametophytic chromosome number as twelve with few doubtful cases; this confirms counts made at meiosis.

In order to effect fertilization, the pollen tube must grow to a total length of more than 1500 microns, that is, 1200 microns or more through the peculiar micropylar channel which Lawson (1909) has described and figured, and 300 microns through the tissue of the nucellus. No evidence was found to substantiate Lawson's account of a breakdown of the nucellar apex in advance of the pollen tube; the latter penetrates "firm tissue" in order to reach the megagametophyte.

The pollen tube takes a direct course through the nucellar tissue, passes along the surface of the megagametophyte to the neck of an archegonium through which it penetrates to reach the egg cell. All four pollen-tube nuclei—the two sperms, the tube nucleus, and the stalk nucleus—pass into the egg.

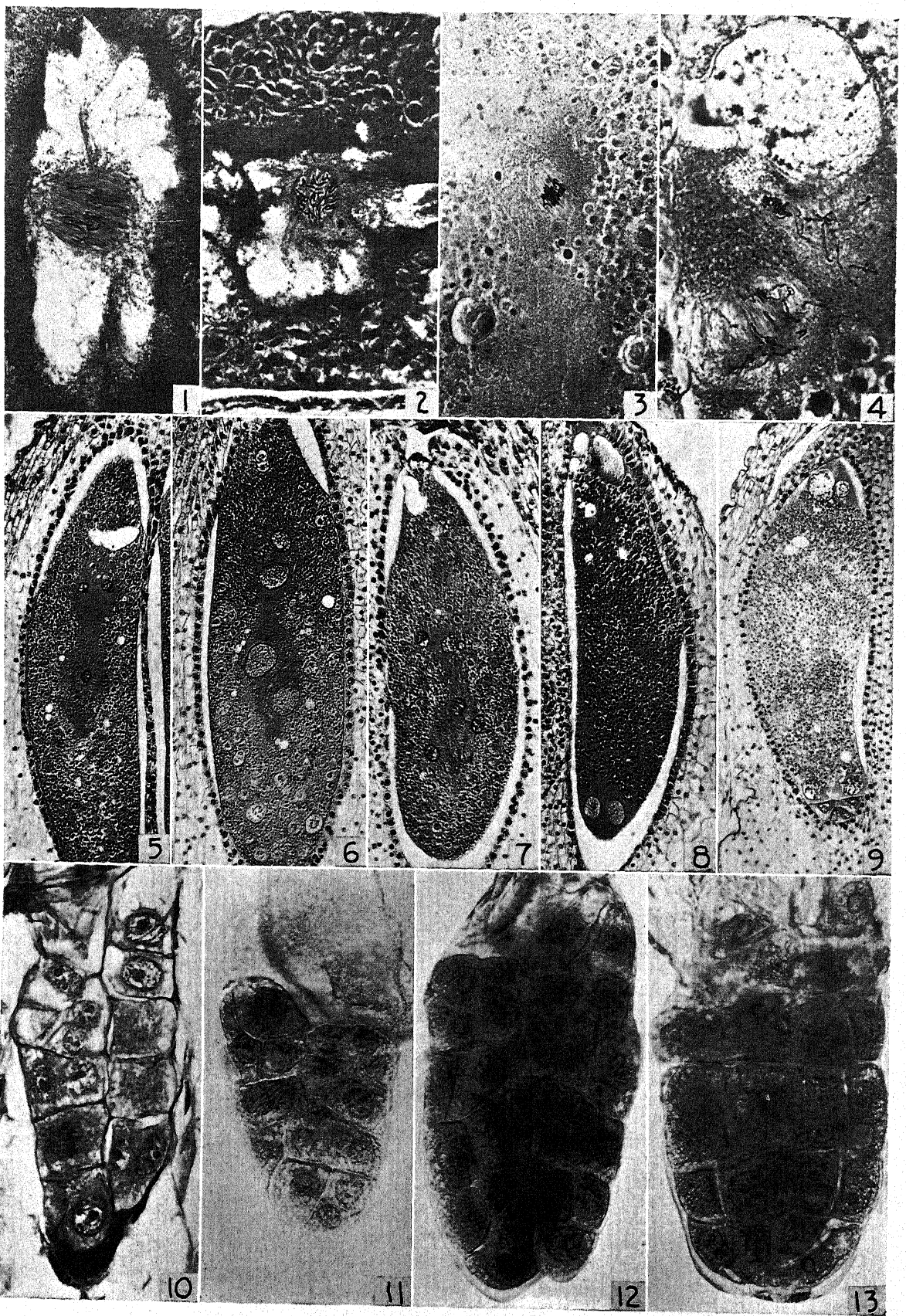
Fertilization occurs between June 1 and June 20, the average varying from year to year in a given locality; in any one year there is a variation of several days between the ovules on an individual tree. Certain trees tend to be much earlier or later than the average and exhibit this behavior consistently.

FERTILIZATION.—As Chamberlain (1935) has remarked, the term "fertilization" may mean entirely different things to different people. The viewpoint here taken is that a description of fertilization in the gymnosperms may include all the relevant details from the time that the pollen tube reaches the egg to the first division of the zygote. Particular emphasis is placed on the fate and behavior of the three "supernumerary" pollen-tube nuclei since this is the basis for the writer's reversal of interpretation of post-fertilization events.

Fate of the pollen-tube nuclei.—Ferguson (1904) described fertilization in *Pinus* in considerable detail and gave particular attention to the behavior of the second sperm, the stalk cell, and the tube nucleus. She found that the stalk cell remains unchanged and finally degenerates; the tube nucleus undergoes various changes and may become smaller or may enlarge considerably. The second sperm may remain unchanged or may divide "amitotically" as described for *Encephalartos* by Arnoldi (1900). Frequently, however, the second sperm may "attempt" to divide mitotically, but Ferguson found no stages beyond late prophase; then activity ceased and degeneration took place. In only one instance was a normal nucleus present in the ventral canal cell; in all others it had aborted or disintegrated by the time that fertilization occurred. Chamberlain (1899) described a very similar behavior in *Pinus Laricio*. That the second sperm may in some instances "fertilize" the ventral canal cell has been reported for gymnosperms (Hutchinson, 1915; Land, 1902). There are several reports of fertilization of the egg nucleus by the ventral canal nucleus (Chamberlain, 1899; Ikeno, 1901; Hutchinson, 1915; Sedgwick, 1924).

There is considerable variation in the number of nuclei which enter the egg. In *Pinus*, however, all four enter (Ferguson, 1904; Chamberlain, 1935) and the same is true of *Taxus canadensis* (Dupler, 1917) and of the Podocarpaceae (Chamberlain, 1935). Apparently it is usual for the supernumerary nuclei left near the neck region of the egg to degenerate promptly (*loc. cit.*). Lawson (1909) stated that in *Pseudotsuga* the entire contents of the pollen tube are discharged into the egg but he did not discuss the fate of the supernumerary nuclei.

The present study has demonstrated conclusively that in *Pseudotsuga* all four pollen-tube nuclei usually pass into the egg; of these, two are sperms, one the stalk cell or nucleus, and the other the tube nucleus. The larger sperm moves rapidly toward the egg nucleus and syngamy takes place. The remaining supernumerary nuclei are often left close to the neck but may be found at any point between the latter and the zygote. Figure 6 illustrates the usual situation in which three supernumerary nuclei are present. The latter may behave in several ways: 1. They may disorganize during a period of several days. 2. They may fragment "amitotically" and so increase the number of free nuclei in the neck region. 3. They may divide mitotically to double or


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quadruple their number, or one or two only may so divide (see fig. 3). Cytokinesis may occur (Allen, 1943, fig. 18 and 32). 4. They may fuse (fig. 4) to form larger nuclei which gradually degenerate. Such "fusions" may take place after mitotic divisions have occurred.

The ventral canal nucleus may enter into these divisions or fusions or may undergo independent divisions within its own cell wall (Allen, 1943). As far as could be determined, there is no "normal" behavior. Table 1 lists the variations observed in a sample of 430 archegonia in which the supernumerary nuclei could be counted accurately.

Although many ovules containing early embryos were examined, the supernumerary nuclei in many of the archegonia had already begun to disorganize. The few cases (32) above enumerated are those in which the nuclei could still be clearly recognized. For this reason the sample may not be truly representative of the overall behavior. It does show a trend, however, similar to that indicated by the more valid data given for the earlier stages.

Chromosome counts in the dividing nuclei near the neck region confirm the interpretation that these nuclei are gametophytic in origin and not sporophytic. They may be derived either from the pollen tube or from the ventral canal cell; the latter possibility is ruled out in many cases by the presence of a recognizable ventral canal cell or its disorganized remains.

There is some evidence that "fusions" between various of the free nuclei in the upper half of the egg may occur. Accurate chromosome counts were not possible but in certain enlarged nuclei more than the haploid number were observed. It may be significant that in many of the eggs with less than three supernumerary nuclei, the latter were abnormally large; this may be the result, however, of simple enlargement which takes place to a degree in the case of all free nuclei within the egg. It does not seem possible, on the basis of the evidence at hand, to relate these "fusions" to "double fertilization" or "triple fusion" in the angiosperms.

Of 29 observed cases of mitoses among the supernumerary free nuclei, 69 per cent occurred simultaneously with the second division of the zygote. Divisions of the nuclei within the egg may, therefore, be related in some way to the conditions existing in the egg at that time. The remaining 31 per cent took place at various times between the 4-nucleate and the 12-celled proembryo stages.

TABLE 1.

Stage	Number of supernumerary nuclei: Less than three	Three	More than three
	per cent	per cent	per cent
From syngamy to the beginning of the second division (171 eggs)	19	75	5
Proembryo stages (227 eggs)	18	46	37
Early embryo stages (32 eggs)	25	31	44

Analysis of *all* the archegonia studied brings out the interesting fact that, in 66 per cent of the cases, the extra supernumerary nuclei have arisen from mitoses which occurred simultaneously with the second sporophytic division.

The main conclusion to be drawn from the observations recorded above is that the fate of the supernumerary pollen-tube nuclei is quite variable, that there is no normal behavior. Mitoses among the free nuclei are quite common; some of them may fragment and fusions may occur the significance of which is unknown. This varied behavior is responsible to no small degree for the previous interpretation of the events which follow syngamy (Allen, 1943).

Syngamy.—Conjugation of the two gametes in *Pseudotsuga* takes place in a manner quite similar

Fig. 1–9. Longisections through the egg.—Fig. 1. ×254. The two sets of chromosomes lie side by side within the highly vacuolate egg nucleus. The paternal chromosomes are above the maternal, that is, closer to the micropylar end. Note the multi-polar spindle and the finely granular cytoplasm which surrounds the egg nucleus. June 16, 1943. —Fig. 2. ×254. A polar view of the chromosomes at syngamy; 24 chromosomes are arranged on the equatorial plate. Note the suggestion of a loose pairing. June 16, 1943.—Fig. 3. ×254. Mitosis of a pollen-tube nucleus in the neck region at the time of the second sporophytic division. June 17, 1943.—Fig. 4. ×440. Free nuclei in the neck region. There is some suggestion that a "fusion" may be occurring. June 6, 1941.—Fig. 5. ×80. Two of the three pollen-tube nuclei and two of the four proembryo nuclei are visible. In the adjacent sections are contained one pollen-tube nucleus within the cytoplasmic sheath and two other proembryo nuclei.—Fig. 6. ×80. Two proembryo nuclei lie distal to the neck of the egg and three pollen-tube nuclei are visible closer to the neck region. No other nuclei are present in the egg except that of the ventral canal cell which does not show in this section.—Fig. 7. ×80. All three pollen-tube nuclei are present together with three of the four proembryo nuclei. At the neck are the remains of the ventral canal cell. No other supernumerary nuclei are present in adjacent sections.—Fig. 8. ×80. Four-nucleate stage of the proembryo just prior to the third division; only two nuclei are visible.—Fig. 9. ×72. Completed proembryo consisting of three tiers of four cells in each. Note the supernumerary pollen-tube nuclei in the neck region.—Fig. 10–13. Longitudinal views of early embryos. ×336.—Fig. 10. Section of a young embryo deep within the megagametophyte. Two of the four embryo units are seen in this section, one apparently ahead of the other. Note the recent longitudinal divisions.—Fig. 11. Dissected embryo showing type B development. Note that the unit at the right has overtopped the other.—Fig. 12. Embryo of type A with four more or less equal units contributing to the whole embryo.—Fig. 13. Embryo of type A. Note the uniform development of the two units visible and the recent division of their terminal cells. It is likely that the latter are giving rise to the group of apical initials which will supersede them.

to that described for other members of the Abietaceae (Ferguson, 1904; Haupt, 1941). A constant feature observed in *Pseudotsuga* is a flattening of the egg nucleus on the side nearest the approaching sperm, a behavior noted by Ferguson (1904) for *Pinus*. The sperm nucleus gradually "sinks" into the egg nucleus but retains its identity for some time; its stainability decreases as Haupt (1941) described for *Pinus*. Finally the membranes between the two gametes disappear and simultaneously the two groups of chromosomes become evident, the paternal lying near the surface of the egg nucleus and the maternal lying near the center. The two sets of chromosomes move toward each other (fig. 1), those of the sperm moving more rapidly so that they come together just above the center of the nucleus. During the movement, spindle "fibers" become visible in connection with each genome; no evidence could be found to suggest that the fibers originate, as suggested by Lawson (1909), from the cytoplasmic masses which form invaginations in the surface of the egg nucleus. The two spindle figures come together laterally to form a common multi-polar spindle with its main axis usually perpendicular to the long axis of the egg. The multi-polar spindle figure appears to contract and become diarch by the time of the first metaphase.

Very little is known, unfortunately, regarding the behavior of gymnosperm chromosomes at syngamy. The most detailed account is that given by Hutchinson (1915) for *Abies* (see also Chamberlain, 1935, p. 336–340). Efforts to confirm these findings have not been successful to date (Beal, 1934; Haupt, 1941). Hutchinson described a pairing of paternal and maternal chromosomes as the two spindles united, followed by an intertwining of the members of each pair; the chromosomes then split transversely to form "tetrads" of daughter chromosomes, two of which pass to each of the daughter nuclei. Probably the most significant feature of this account is the pairing of "homologous" chromosomes of the two parents, a behavior duplicating that which takes place in meiosis. More recent work on fertilization in *Pinus* (Beal, 1934; Haupt, 1941) has not confirmed Hutchinson's interpretation for *Abies;* pairing was not observed and the segmentation of the chromosomes was longitudinal. Chamberlain (1916), however, was able to find only the gametophytic number of chromosomes at metaphase of the first sporophytic division in *Stangeria;* he has suggested (1935) that this might be explained on the basis of a pairing of chromosomes as described by Hutchinson since the latter found the haploid number of *pairs* in *Abies*. It is evident that, if such pairing does occur, it must be a fleeting phenomenon as it is in meiosis proper but without the polar separation of homologous units. Critical work from which definite conclusions could be drawn would undoubtedly require very closely spaced collections during the time of fertilization with immediate dissection and killing in the field. The work of Beal (1934) and of Haupt (1941) on *Pinus* cannot be accepted yet as final proof that pairing does not take place in the gymnosperms.

Many preparations of *Pseudotsuga* ovules at stages of fertilization were examined carefully. Of these, many showed the functional sperm in contact with the egg nucleus, a considerable number showed the two spindle figures lying side by side within the egg nucleus, but only two contained the critical stage in which the 24 chromosomes are arranged on the equatorial plate (fig. 2). In these there seemed to be a tendency for chromosomes of similar size to lie together loosely. It is possible that they represented stages just prior to actual pairing.

As will be described, the later stages of embryogeny in *Pseudotsuga* and in *Larix* (Schopf, 1943), and possibly in other conifers such as *Pinus*, suggest that there may be a genetic difference between the first two derivatives of the zygote and between their progeny. Such could be explained on the basis of a pairing of chromosomes at syngamy. This is, of course, a highly speculative thought and is offered only as such. It is of such theoretical interest, however, that it is to be hoped that an intensive study of syngamy in the gymnosperms may be undertaken in the near future.

THE PROEMBRYO.—Since there is no actual "fusion" of the two gametes during syngamy, it is not possible to define the zygote as a single nucleus. The chromosomes of the egg and of the sperm arrange themselves immediately on the equatorial plate in preparation for anaphase of the first division. The proembryo is therefore considered here to include everything from the two-nucleate stage. resulting from the first division, to the 12-celled stage of the completed proembryo. The proembryo phase ends when the primary suspensor cells elongate and the embryo tier breaks through the base of the egg into the gametophyte tissue.

As in most gymnosperms, the proembryo passes through a free-nucleate phase. Chamberlain (1935) contended that the early nuclear figures are too small to segment the large mass of cytoplasm within the egg and Schopf (1943) agreed with this hypothesis. Such may be a possible explanation but it is important to note that in many angiosperms there are no walls formed for some time between the endosperm nuclei, even in the very narrow passage between the embryo and the embryo-sac membrane. Bailey (1920) has shown that the young cell plate formed in the fusiform initials of the vascular cambium may attain a remarkable size. Sharp (1911, plates 11 and 12) has described and figured the first division of the endosperm nucleus in *Physostegia* which is accompanied by the formation of a longitudinal wall through the embryo sac. Furthermore, in *Pseudotsuga,* cytokinesis may occur frequently between the free nuclei in the fertilized egg cell (Allen, 1943). It is tempting to suggest that cytokinesis is induced by factors inde-

pendent of the spatial relationship, that these factors may affect other nuclei within the egg and induce cell-plate formation between them.

The first two divisions of the zygote result in the formation of four free nuclei near the center of the egg (fig. 5 and 6). At first these nuclei are small and consist mainly of chromatin but they enlarge quickly as they move towards the basal end of the egg (fig. 7 and 8). Throughout development the zygote and its early derivatives are surrounded by cytoplasm of regularly granular appearance (see particularly fig. 2, 5, and 8). As in the case of the nucleus of the mature egg, this may again represent a region of digestion lying between the heterogeneous food reserve of the egg and the growing sporophytic nuclei. During the next division of the proembryo nuclei, after which cytokinesis takes place, the eight nuclei are very small and deep staining, but they enlarge rapidly following formation of the cell plate. The first wall formed in the proembryo is always transverse to the long axis of the egg as it is in the great majority of the archegoniates, and is followed by the formation of two axial walls (Allen, 1943). The result is a two-tiered proembryo the upper cells of which are open to the egg. The lower tier divides by transverse walls to complete the proembryo of three tiers, the suspensor tier being interposed between the open or "relict" tier (Schopf, 1943) and the embryo tier which lies distal to the micropyle (fig. 9). A rosette tier, formed by the division of the cells of the open tier, has not been observed in the material studied. Lawson (1909) described four tiers in the proembryo of *Pseudotsuga* but Buchholz (1926, 1931) was unable to find true rosette cells in this conifer.

Discussion.—The phylogenetic meaning of the free-nuclear phase in gymnosperm embryogeny is uncertain. Free nuclear division is characteristic of many primitive algae and fungi (Fritsch, 1935) and of many highly protected sporangia and gametophytes with rich stores of food, where divisions are rapid and nuclear migrations occur. Similar conditions are present in the egg cytoplasm of conifers. Whether or not the 8-celled proembryo of *Pseudotsuga* is equivalent to the 8-celled stage of an angiosperm embryo or of a lower tracheophyte embryo is problematical and at present not answerable. Schopf (1943) considered that the ". . . . primary embryonic polarity in gymnosperms is delayed in appearance . . . ." in comparison with that in the vascular cryptogams. Schopf concluded that ". . . . the first definite polarity indication of these embryo-forming units in *Larix* and others of the Pinaceae is observed in the *last* cell division within the proembryo," and that this segmentation of the apical tier is perhaps ". . . . functionally equivalent to primary segmentation of the zygote in vascular cryptogams." Perhaps this viewpoint may be attributed to Schopf's conception of cleavage polyembryony in conifers as a *primitive* behavior; if cleavage is a derived condition, this viewpoint is hardly tenable. Similarly, Schopf's concept does not apply to the cycads in which over one thousand free nuclei may be present in the proembryo prior to wall formation (Chamberlain, 1935). It seems more reasonable to consider the first (transverse) wall in the proembryo of *Pseudotsuga* as *functionally* equivalent to the primary segmentation of the zygote in the lower tracheophytes. This wall is constant in its orientation and in its time of appearance, and seems to be a true indicator of polarity. The basic problem relative to the free-nucleate phase is, however, still awaiting clarification. The term "polarity unit" which Schopf (1943) used in discussing the embryogeny of *Larix* cannot be applied to gymnosperms, such as *Pseudotsuga, Ginkgo,* and the cycads, which exhibit simple polyembryony. If used in a very broad sense, it would refer to the entire walled embryo and would have no particular usefulness.

In his intensive study of the embryogeny of *Larix*, Schopf (*loc. cit.*) described an important event which takes place during proembryo development, an event which predetermines to a considerable extent, in his opinion, which of the four "polarity units" will be repressed during the subsequent two weeks. Each "polarity unit" in *Larix,* namely, each embryo cell with its suspensor cell, has the potentiality of becoming a mature embryo, but normally only one of the four will survive. Schopf came to the conclusion that the *position* which each embryo cell occupies in the base of the egg more or less predetermines its fate: the two cells which occupy the broad diameter of the elliptical egg are favored over the other two. Schopf stated (p. 28) "In later growth the slight initial advantage for the larger cells becomes increasingly important with reference to selection of the single successful polarity unit."

Schopf's conclusion is difficult to accept completely on the basis of the evidence at hand. In the first place, the embryo cells are "confined" within the base of the egg for not more than two days and for possibly less (Schopf, 1943, p. 15); precise and convincing evidence is desirable to show that the environment during this short period can affect later development so regularly and fundamentally. Schopf based his hypothesis upon the differences in transverse sections in the size of the four embryo units from the same zygote; in his "typical" illustration (p. 28, fig. 8b), however, the two units occupying the broad sides of the ellipse actually appear to be *smaller* than the other two and to have smaller nuclei. It is evident that measurements of cell volume from serial sections would be necessary for an accurate comparison of the size of the embryo cells.

Although a similar arrangement is frequently found in proembryos of *Pseudotsuga,* it is by no means universal. Often the four embryo cells occupy approximately equal quadrants of the sectionally elliptical base of the egg.

Whatever may be the nature of the factors which cause early repression of certain embryo units in

Larix, Schopf has brought into the open this very complex problem, namely, that three of the four embryo units resulting from each zygote are regularly eliminated early in embryogeny. This is a remarkable behavior: four *apparently* equipotential units, surrounded by abundant food reserves, compete so vigorously that within two weeks three of them are repressed and disappear.

It is difficult to agree with Schopf that the dominant "polarity unit" can be recognized at the early stages shown in his figures 17 and 17a (p. 30). Although cell "1" projects slightly ahead of the others and alone is non-vacuolate, these characteristics seem poor evidence of superiority. Bailey (1930) has shown that extreme vacuolation is characteristic of active cambial initials. Preparations of *Pseudotsuga* occasionally show all four terminal embryo cells highly vacuolate and the presence of vacuoles is hardly an indication of repression. It is evident that the factors which govern the elimination of certain embryo units still remain to be investigated carefully.

It was suggested in the previous section that there may be a genetic difference between the two nuclei resulting from the first division of the zygote, and between their derivatives. This suggestion was based upon the possibility of pairing and fortuitous grouping of homologous chromosomes during syngamy and the first sporophytic division. This does not explain, any more than does Schopf's hypothesis, how one "superior" unit could finally suppress the other in *Larix,* but this speculation may be worthy of consideration in future work. Development in general depends upon both genetic and environmental factors and without conclusive evidence one cannot be casually eliminated in favor of the other. There is evidence in *Pseudotsuga* during early embryogeny that normally two of the four embryo cells of the proembryo are "superior" to the others and that the embryo from each zygote may consist almost entirely of cells derived from the two "superior" units.

It should be pointed out that in *Larix* (Schopf, 1943), the primary suspensors disintegrate very early and that, therefore, the tracing of individual "polarity units" to their original egg is a doubtful procedure. Other conifers which exhibit cleavage polyembryony, such as *Pinus* or *Tsuga* (Buchholz, 1931), or *Abies* (Hutchinson, 1924), might provide suitable material for further investigations of this interesting problem.

THE EMBRYO PROPER.—In his recent monograph on the embryogeny of *Larix,* Schopf (1943) introduced a new terminology and recognized four stages: "pro-stage," "meta-stage," "ana-stage," and "telo-stage." "Pro-stage" is synonymous with the proembryo phase and seems unnecessary. "Meta-stage" ends at the time ". . . . when the embryo anlage becomes massive," which seems somewhat indefinite. Division of late embryogeny into "ana-stage" and "telo-stage" has more to commend it but the beginning of "ana-stage" is necessarily indefinite. It seems desirable to divide embryogeny artificially in such a way that the division is as natural as possible and that other groups of plants may be described under the same headings; the scheme should certainly be applicable to other gymnosperms and, if possible, to angiosperms as well. Schopf's proposal may be useful for many conifers but fails when applied to *Ginkgo* and to the cycads. In order to avoid difficulties of this kind and to make the division as objective as possible, embryogeny has been divided into two stages: *early* and *late.* These two phases of development are "separated" by one of the outstanding features of ontogeny, namely, the appearance of the generative meristem of the root. This natural feature of embryogeny of all gymnosperm embryos seems to offer a satisfactory separation point, prior to which the embryo is undifferentiated and massive, and following which it shows differentiation into various regions and a foreshadowing of its later cellular organization. Any separation of a continuous developmental process is artificial, but in this case is justified by rendering easier the task of description; yet it does not lead to complexity as further subdivision might. A more detailed separation into sub-stages may be desirable for minute comparisons of species and genera but can be based upon the above divisions.

The early embryo.—A brief summary of early embryogeny in related conifers seems desirable at this point as a basis for a discussion of the *early* stage in *Pseudotsuga.* Both simple and cleavage polyembryony occur in the Abietaceae and Buchholz (1931) has compared most of the genera from this standpoint. *Pinus* represents a genus in which cleavage appears to be a constant feature though variable in its time of occurrence; in Jack pine (*Pinus banksiana* Lamb.), for example, the four embryo units separate or cleave soon after elongation of the primary suspensors, and the four young embryos become virtually independent of one another. Often associated with cleavage polyembryony is the potentiality of the rosette cells to divide and produce small embryos which soon abort. On the other hand simple polyembryony, as exemplified by *Picea* (Buchholz, 1931), does not involve cleavage of the embryo group into four separate units; instead, all units remain united and together develop into one embryo. Simple polyembryony involves, therefore, the production within one ovule of several embryos each derived from one complete zygote. Cleavage polyembryony, in contrast, results in the production within one ovule, of several zygotes each of which by cleavage may give rise to several potential embryos. Under the group showing cleavage Buchholz (1931) included *Pinus, Cedrus, Tsuga,* and *Pseudolarix,* and under simple polyembryony included *Abies, Picea, Larix,* and *Pseudotsuga.* In his recent investigation Schopf (1943) demonstrated that *Larix* exhibits *delayed* cleavage polyembryony, the separation of the individual units occurring much later than in *Pinus.*

Schopf has suggested that the other genera ". . . . previously thought to possess simple polyembryony may very likely be similar to *Larix* in this respect." Buchholz (1942), however, stated that "simple polyembryony prevails in *Abies venusta* and is the normal condition in all firs that have thus far been examined" (see Hutchinson, 1924, on *Abies*).

As far as could be determined in this investigation, the derivatives from one zygote in *Pseudotsuga* together develop into one embryo. "Delayed cleavage," as observed consistently in *Larix* (Schopf, 1943), or occasional cleavage as reported for *Abies* by Hutchinson (1924), have not been observed. Behavior is not stereotyped, however, and a considerable variation occurs such that it is not possible to give an accurate statistical account of the "normal" behavior. Some two hundred dissected embryos and over one hundred sectioned embryos, in various *early* stages, form the basis for the following conclusions.

Type A. Each of the four "embryo units," which together were derived from a single zygote, may contribute more or less equally to the developing embryo. Each unit has its own terminal cell which adds to the individual filament by transverse divisions. The terminal cells eventually divide longitudinally or obliquely to establish a group of apical initials such as occur at the shoot apex of many gymnosperms (Foster, 1941). These initials then contribute to the entire embryo by means of anticlinal and periclinal divisions and eventually give rise to the shoot apex of the embryo. In this type of development, which is relatively common, there is at no time an apical cell which dominates the entire embryo and which is typical of the young embryo of *Pinus* (Buchholz, 1918). This type of development is illustrated in figures 18a, 18b, 19, 12, and 13.

Type B. The most common program involves the attainment of dominance by two of the terminal cells by a gradual process of overtopping. The dominant pair soon acquires two cutting faces and adds to all four embryo units for a limited period. Finally the terminal cells give rise to a group of apical initials with a final result similar to that of the first type. In this case, however, the initial group, and hence ultimately the shoot apex, are derived from only two of the original four embryo cells of the proembryo. Representative examples of embryos following this type of development are shown in figures 10, 14, 15, 16, 17, 21, and 22.

Type C. In a few instances there is evidence that one terminal cell may dominate the other three by gradually overtopping them and acquire three or four cutting faces. The terminal cell is then truly an "apical cell" (Schuepp, 1926; Johansen, 1945) such as occurs in the embryo of *Pinus* (Buchholz, 1918). Presumably the behavior of the apical cell resembles that of *Pinus* in giving rise to a group of apical initials. The evidence for this type is not conclusive and further investigation is desirable. Examples of this type are illustrated in figures 20a and 20b.

The most common program of development (type B), in which two units overtop the other two, would probably be explained by Schopf (1943) on the basis of the unequal position of the four original embryo cells in the base of the egg; this would not explain the relatively common condition in which all units contribute equally to the embryo, as reported also for *Picea* (Buchholz, 1931) and for *Abies* (Hutchinson, 1924; Buchholz, 1942). Schopf's hypothesis may be correct, but as suggested, there may be a genetic difference of varying degree between one pair of embryo units and the other.

It is difficult to see how competition between "equivalent" derivatives of one and the same zygote could have survival value; indeed, it would appear to be disadvantageous if it retarded the development of the embryo. On the other hand, competition between "unequal" derivatives might represent a special type of selection having survival value. On this basis, cleavage polyembryony, which results (in *Pinus*) in the early "freeing" of the "superior" units, may have selection value and may, therefore, represent a derived condition. This is admittedly speculative, but the following evidence is worthy of consideration.

Buchholz (1926) argued that simple polyembryony has survival value lacking in cleavage polyembryony because of the more massive embryo and stiffer suspensor in the former case. Schopf (1943) agreed that simple polyembryony is derived, yet he showed that delayed cleavage in *Larix* subjects the individual embryo units to more intensive intraembryonal competition; this slows growth to such an extent that "meta-stage" is ". . . . two to four times as long in *Larix* as it is in *Pinus*" and may be even longer. This same stage in *Pseudotsuga* occupies a length of time similar to that in *Larix* (about two weeks) but there is considerable variation which seems to be related to the type of development (A or B). Thus, embryos of type A were in most cases past Schopf's "meta-stage" by the end of June whereas many embryos of type B were much smaller and comparable to *Laris* embryos after a similar period of time. Statistical data are not available, however, to indicate whether or not this apparent difference is real.

It seems possible that type A represents the development of an embryo in which there is no effective difference in rate of growth between the four units, and that type B represents the behavior of an embryo in which two units are superior to the other two. When all units are equipotential, they combine more or less equally to produce an embryo; when two units are superior to the others, they are the ones which give rise to the apical initials. Thus, in the latter case, most of the embryo, including the shoot apex, is derived from the dominant embryo units; the two "inferior" units apparently contribute mainly to the suspensor system. In

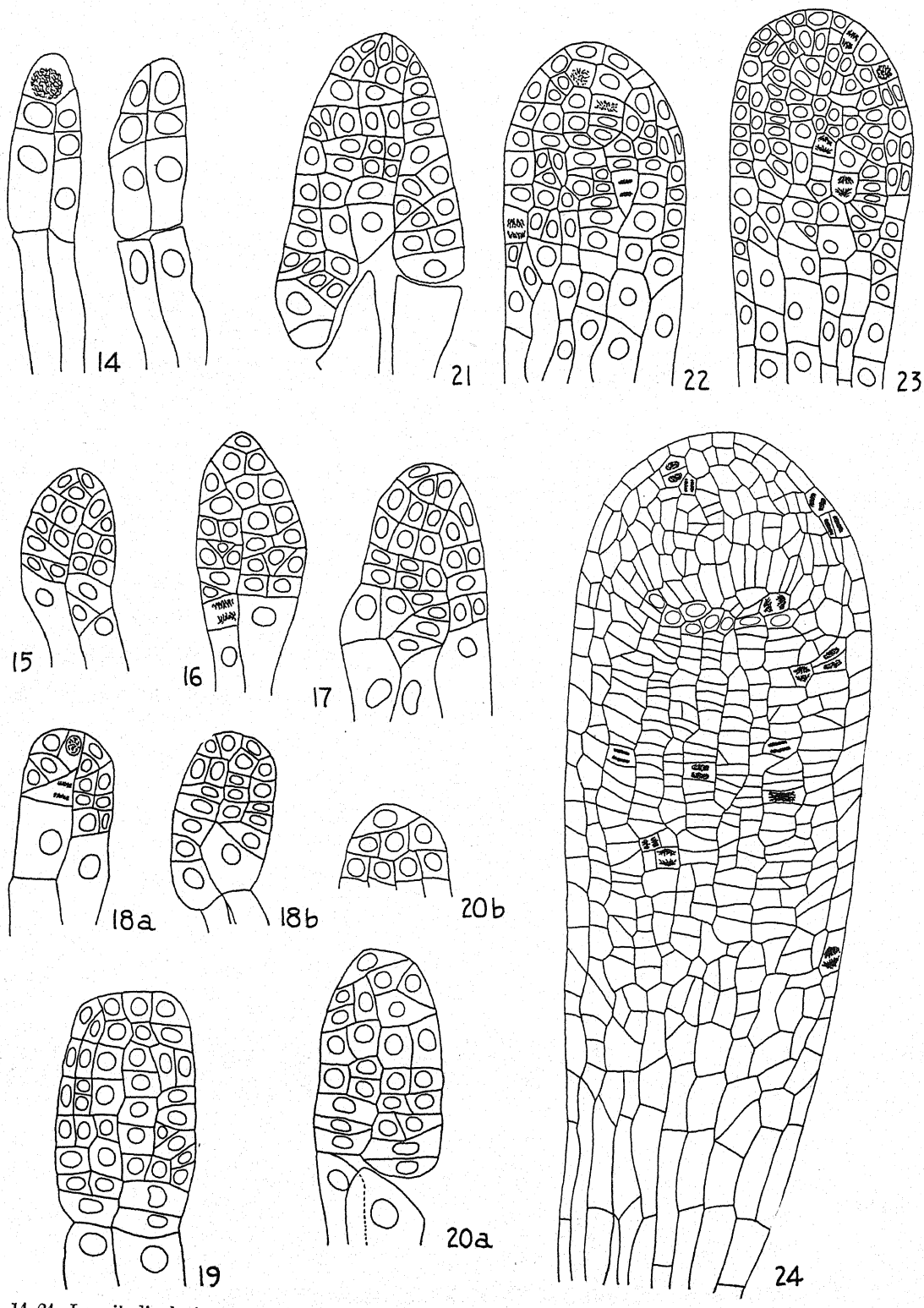


Fig. 14–24. Longitudinal views of embryos during the *early* stage. ×200.—Fig. 14. Two views of a very young sectioned embryo. Left: surface view showing one dominant unit and one repressed unit. Right: the other two units of approximately equal size. Probably type B.—Fig. 15. Lower view of a type B embryo in which the left unit has overtopped the right. The surface view showing the other two units is very similar.—Fig. 16. Surface view of a type B embryo in which the unit to the right is dominant. The lower two units are similar to the pair shown.—Fig.

Pinus and in *Larix*, due to cleavage, the dominant embryo unit produces the entire embryo and the repressed units are completely excluded.

Whatever the nature of the factors deciding the part which a given embryo unit will play, whether they are genetic or fortuitous, in extreme cleavage polyembryony there is only a short period of "competition" before the units are freed to continue development at their own individual rates. In simple polyembryony, at least in *Pseudotsuga*, the units remain together and presumably their growth is retarded until the dominant units gain the ascendency; if the embryo units are more or less equipotential, the entire embryo forges ahead at its maximum rate.

Buchholz (1926) suggested that cleavage in the early days might have represented ". . . . an adjustment which restored a highly effective form of developmental selection to the life cycle." He suggested further that in the elimination of other embryos ". . . . chance plays a minor role, for the persisting embryo usually merits this reward," and that the differences which are inherent among the several embryos from the same zygote ". . . . may have their origin in a *chromosomal re-assortment of hereditary qualities during the first few cell divisions of the zygote*, as was observed by Hutchinson (1915) in *Abies*. . . ." Buchholz stated further that "Where cleavage polyembryony is involved, the competition measures the best pollen tube plus *the best fraction of its zygote*, against many other similar combinations within the same developing seed." It seems logical to carry this reasoning to the conclusion that an embryo consisting entirely of the "best fraction" of the zygote might compete successfully against a more massive (at first) embryo made up of both the "better" and the "poorer" fractions of the zygote. Buchholz (1926), however, argued that in a single ovule, the massive embryo produced by simple polyembryony would always win out over the smaller embryo resulting from cleavage, and hence that simple polyembryony must be a *derived* condition. The writer is forced to conclude that the relation between simple and cleavage polyembryony is as yet obscure and that a better understanding of the problem may come from an exhaustive enquiry into the early phases of embryogeny in the Abietaceae, especially into the cytology of syngamy and proembryo development. The very precision of cleavage in *Pinus* as compared with simple polyembryony seems *a priori* to suggest that cleavage is a specialization, particularly if it can be shown that one fraction of the zygote is superior to another as suggested by Buchholz (1926) and by Schopf (1943), and indicated in the present investigation. It is noteworthy that Hutchinson (1924) concluded that *modified* cell divisions in the proembryo of *Abies* result in cleavage and that cleavage is probably a derived characteristic.

Once a group of apical initials has appeared (fig. 19, 21, 22, and 23), no matter what the type of earlier development, the young embryo of *Pseudotsuga* behaves much like that of *Larix* (Schopf, 1943). The cell pattern varies considerably but continued growth produces a club-shaped embryo very similar to that of *Ginkgo* or of the cycads (Chamberlain, 1935) (fig. 24). At no time is the embryo or any part of it invested with a dermatogen; surface cells exhibit numerous periclinal divisions throughout embryogeny.

An outstanding evidence of polarity is the acropetal development of a rib meristem continuous with that of the suspensor system; it extends from the latter to a point some eight to ten cells away from the free apex of the embryo, a point where a small group of cells behaves independently of the rib meristem and later becomes the generative meristem of the root (fig. 24). The small spherical region between the rib meristem and the apex has been called the "pleromic centrum" by Schopf (1943) and represents the earliest recognizable stage of the embryo stele. In order to avoid histogen terminology, this region might be termed appropriately the "stele promeristem"; however, no exact predestination is implied since it is realized that cells of this primordial region may give rise in varying degree to cells or tissues other than those of the stele. The embryonic stele of the dormant embryo does arise in the main from the stele promeristem.

At the end of the early stage, the young embryo consists of the stele promeristem delimited at one end by the free surface which will give rise to the shoot apex of the mature embryo, and at the other end by a root generative meristem in its youngest recognizable stage (fig. 24). Behind the latter is a massive zone of rib meristem which is continuous with the suspensor system. The cell pattern which

17. Surface view of a larger type B embryo. Note the considerable bulk of the left hand unit. The lower pair not shown are very similar to these.—Fig. 18. Two views of a type A embryo. a. surface pair of units; b. lower pair of units. Note the relatively equal size of the four units.—Fig. 19. An older type A embryo in surface view. All four units are apparently contributing equally.—Fig. 20. Two views of a type C embryo in which there is one dominating terminal cell or apical cell. a. Lower pair of embryo lineages; b. upper pair of lineages having the same apical cell as in a.—Fig. 21. Older embryo of type B.—Fig. 22. Median longisection of an older embryo, probably type B.—Fig. 23. Median longisection of an embryo near the end of the *early* stage; type uncertain but probably type A.—Fig. 24. Median longisection of an embryo at the end of the *early* stage. The nuclei of the root generative initials are outlined. Between the initials and the free apex lies the spherical *stele promeristem;* surrounding the stele promeristem is an irregular tissue designated as the *cortex promeristem.* Between the initials and the suspensor system lies the massive *rib meristem* from which is derived in great part the root cap of the older embryo. Note the evidence of periclinal divisions in surface cells.

is now evident foreshadows to a great extent the future course of embryo development.

SUMMARY

An intensive study of several hundred archegonia of *Pseudotsuga* has provided evidence that proembryo formation is essentially similar to that in *Pinus* with the exception that the completed proembryo of the former consists of only three tiers.

All four pollen-tube nuclei are discharged into the egg; three of these, the "supernumerary nuclei," may behave in various ways. They may disorganize, they may fragment "amitotically," mitoses may take place often accompanied by cytokinesis, they may "fuse" with one another and finally degenerate. Apparently there is no normal behavior and more than three nuclei are commonly present near the neck region of the egg. The irregularity of this phase of development was responsible in the main for the writer's previous interpretation (Allen, 1943).

Although no evidence for a pairing of maternal and paternal chromosomes at syngamy was obtained, in the two preparations showing critical stages of the first metaphase there was an indication of loose pairing of chromosomes of similar size. The suggestion is made that some of the peculiarities of later embryogeny in certain conifers may be a result of pairing of chromosomes at syngamy.

Simple polyembryony occurs in *Pseudotsuga* and there is no evidence of cleavage. All four embryo lineages from the one zygote may contribute equally to the late embryo, or two lineages may overtop the others and give rise to the apical initials. The latter appears to be the more common program. There is some evidence that occasionally one lineage may overtop the other three and that its terminal cell may become the apical cell of the entire embryo. Certain facts support the concept that simple polyembryony is less specialized than cleavage although the reverse viewpoint seems to be generally accepted. It is concluded that the relation between simple and cleavage polyembryony is as yet obscure.

Embryogeny has been divided into *early* and *late* stages, the artificial separation being suggested by the appearance of the root generative initials which set apart the two highly meristematic regions—the stele promeristem, and the massive rib meristem which is continuous with the suspensor system.

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THE ANATOMY OF CIRCUMSCISSILE DEHISCENCE [1]

R. V. Rethke

COMPARATIVELY LITTLE is known about the mechanism of dehiscence in dry fruits of the angiosperms. A number of contributions have been made to the cytology of the mechanism of dehiscence of anthers and of the sporangia of plants below the Spermatophytes, but the bulk of research pertaining to dehiscence of fruits has been done by foreign workers; and little of that is more than gross observation.

According to Saunders (1925), dehiscence in the syncarpous capsule, composed of valve carpels, is septicidal, or sutural. This summarizes all the research done by foreign workers since none of their investigations concerned the pyxidium type which exhibits circumscissile dehiscence. Since capsule dehiscence in this form is not along the fusion lines of the carpels, is not parallel to the main vascular bundles, and is not in the main direction of growth, it is of interest to discover just how dehiscence is effected.

Gregoire (1924) describes dehiscence at some length, both histologically and morphologically; but only in follicles and pods in which the splitting lines are longitudinal and coincide for the most part with previous fusion lines.

Troll (1923) considers various types of dehiscence found among families of both monocotyledons and dicotyledons. He devotes very little of his discussion to histological features, confining himself rather to gross aspects which are externally visible. His point of emphasis, common to all the groups examined, is that the lines of rupture invariably occur in the portion of the capsule which is physically uppermost.

Hofmann (1931) investigating the fruit of *Aspidosperma megalocarpon*, concludes that dehiscence is brought about by stresses set up in the wall of the fruit where bands of sclerenchyma alternate with bands of parenchyma. The parenchyma, with a higher water content than the sclerenchyma, shrinks more upon drying. The resulting tension causes the 3-carpellary fruit to split lengthwise down two sides from apex to base.

GROSS ANATOMY OF PORTULACA GRANDIFLORA.—The mature capsule is ovoid and is divided into two readily discernible portions, the lid and the base. The upper margin of this base is delimited by a prominent ridge. This ridge was produced when the stamens and the perianth dropped from the flower since the ovary is half-inferior. Below the ridge the base of the fruit, composed largely of hypanthium, is partially obscured by floccose hairs.

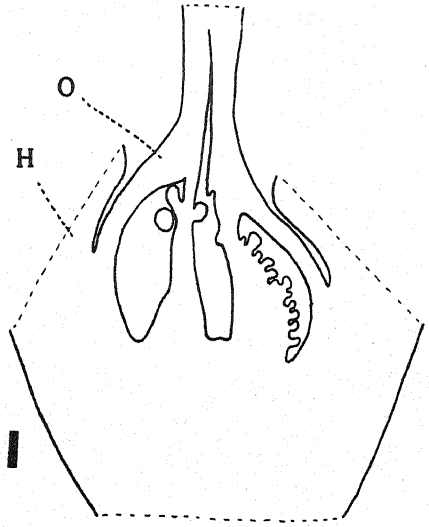
HISTOLOGY OF PORTULACA GRANDIFLORA.—The youngest stages of the ovary examined exhibit no indication of dehiscence. All cells in the wall of the ovary and in the hypanthium with the exception of the future vascular supply are meristematic.

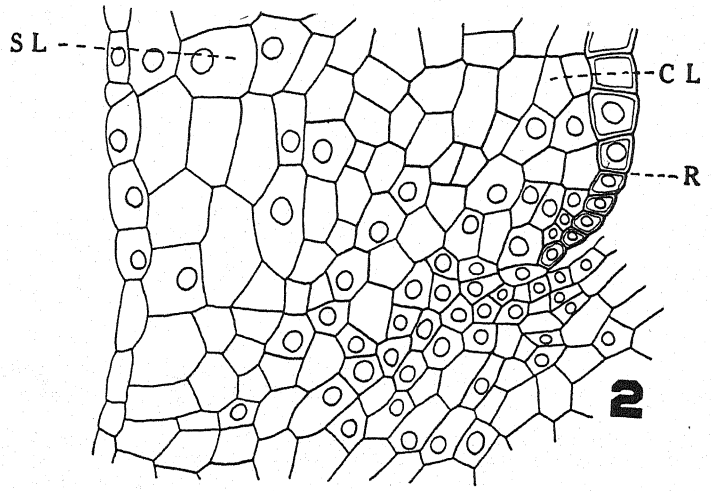
It is not until about the time of the development of integuments that evidence of dehiscence appears. At this stage of ontogeny the ovary wall begins to thicken at about its equator until a pronounced ridge is formed slightly above the level where the hypanthium diverges from the wall of the ovary. Examinations of still older specimens reveal this ridge increasing in prominence as the capsule matures (fig. 2) due to meristematic activity.

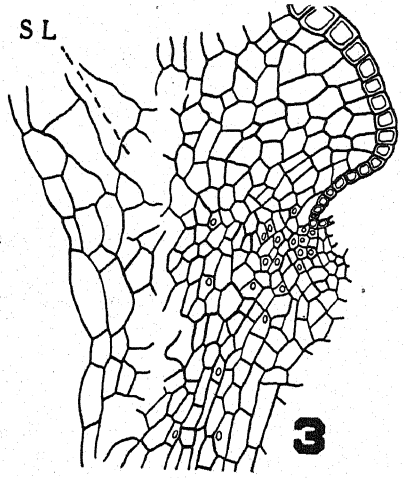
By the time the ridge can be seen with the unaided eye differentiation of cells has also proceeded in other parts of the wall of the ovary. The wall of the ovary (fig. 2) above the divergence of the hypanthium now presents two fairly distinct layers, namely, an outer layer of smaller compact cells and an inner layer of larger specialized water storage cells. The outer epidermal cells of the lid are becoming lignified. The outer epidermal cells below the ridge are much smaller in size than those covering the ridge and the rest of the lid.

Another feature found in older specimens is the general breakdown of cells in the spongy layer of

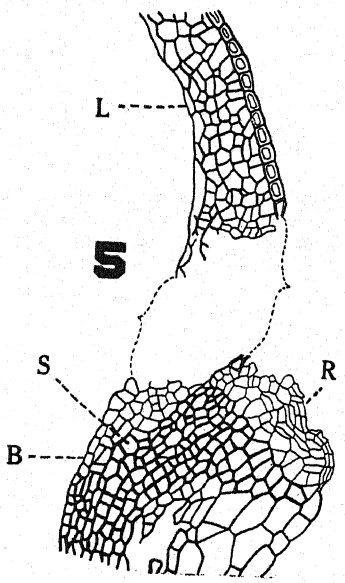
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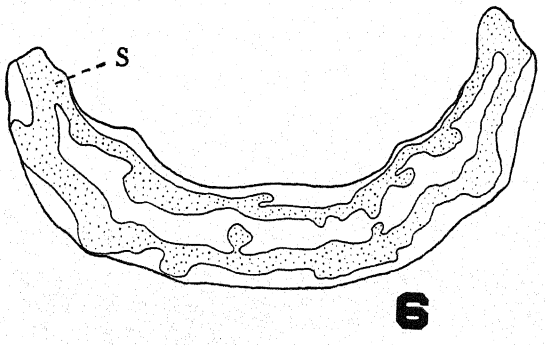

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the wall of the ovary for some distance below the zone of dehiscence (fig. 3) and also in the wall above this level. Rupture, disorganization and resorption of spongy parenchyma is apparently of wide occurrence in dry fruits as True (1893) describes it even in a fruit which never dehisces, the caryopsis.

Shortly before dehiscence occurs all cells in the zone of dehiscence, except the inner and the outer epidermal cells of the wall of the ovary, become completely separated (fig. 4). This rupture seems to be due primarily to the separation of the cells rather than by the disruption or digestion of their walls. The line of dehiscence is rather irregular.

When dehiscence is completed, the lid is much thinner than in earlier stages and is fewer cells in thickness due to the breakdown of cells in the spongy layer. The outer epidermis of the lid is heavily lignified. Sclerenchymatous tissue is abundant in the hypanthium. Lignification is most pronounced in the region of the ridge left when the perianth and the stamens dropped (fig. 5). The sclerenchyma in the remainder of the base conforms to the position of vascular bundles forming an interconnected sheath and network (fig. 6).

Gross anatomy of hyoscyamus niger.—In *Hyoscyamus niger* the mature capsule also consists of two easily distinguished parts, a lid and a base (fig. 7). In this species the lid is much more solidly constructed than the base. There is a ridge present on the upper margin of the base but it was not produced when stamens and perianth dropped as in the half-inferior ovary.

Histology of hyoscyamus niger.—Various series of the young ovary show cells of almost uniform meristematic characteristics with the exception of the procambial strands. Early in the ontogeny of the ovary though, the upper carpel wall, which later develops into the lid, becomes noticeably thicker than the rest of the wall (fig. 8).

The first indication of dehiscence is a slight difference in the thickness of the wall of the ovary in the zone in which splitting later occurs (fig. 9). Microscopically two circumscissile ridges form with a very shallow groove between them. The cells near these two ridges are meristematic while cells in the rest of the wall of the capsule now are attaining the appearance of parenchyma.

As the ovary matures, the lid increases in thickness. The outer and the inner epidermis in the base become thickened due to the deposition of lignin. In the lid only the walls of the outer epidermis become appreciably thicker. The cells in the immediate region of the zone of dehiscence still exhibit meristematic characteristics. These meristematic cells extend from the outer to the inner epidermis but are more numerous nearer the outer epidermis than the inner (fig. 10). Even though these cells are apparently undergoing mitosis their long axes are directed more or less parallel to the future break. A deep external groove has now been formed slightly below the place of future splitting. Even when the time for rupture approaches, a distinct meristematic region remains across the zone of dehiscence (fig. 11).

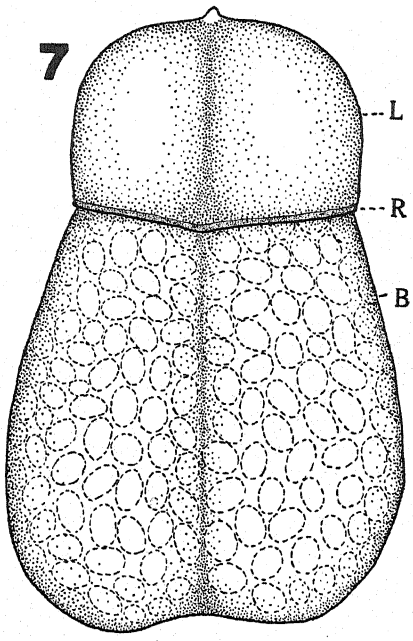
When dehiscence is completed (fig. 12), the lid is seen to be composed of relatively compact tissue, at least near the actual region of splitting. The inner epidermis of the lid and of the base is extremely lignified. There is also an extensive area of lignified cells above the line of dehiscence and several layers of similar cells adjacent to the inner epidermis of the lid. Dehiscence has been effected in meristematic cells and consequently these cells or their parts can be found adjacent to the rupture both in the lid and in the base. In the base are large intercellular spaces confined chiefly to the inner portion of the wall of the fruit. In the base these intercellular spaces do not extend completely to the zone of dehiscence.

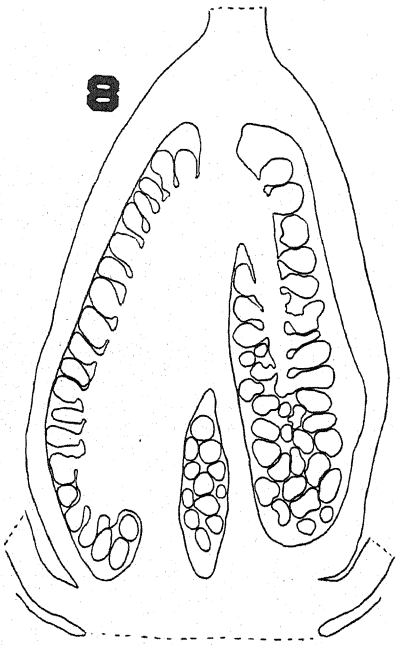
Gross anatomy of plantago spp.—The fruits of different species of *Plantago* are all more or less ovoid, but here the lid and the base are not so readily defined. The lid is somewhat thicker than the base although this is not true in its entirety since cell numbers are approximately the same in the lid as in the base near the zone of future separation. The zone of dehiscence appears as a definite but not too prominent circumscissile line.

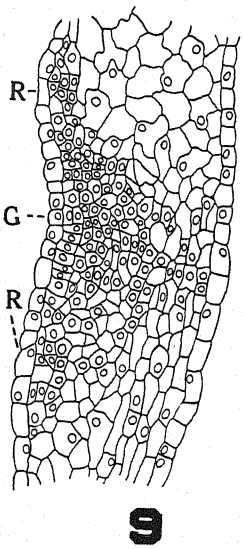
Histology of plantago major.—In longitudinal section the young ovary is a round structure superior to other floral parts, with several ovules in each loculus (fig. 13). It is chiefly made up of meristematic cells, and as in *Portulaca grandiflora* and in *Hyoscyamus niger,* the wall of the ovary exhibits nothing of future dehiscence (fig. 14).

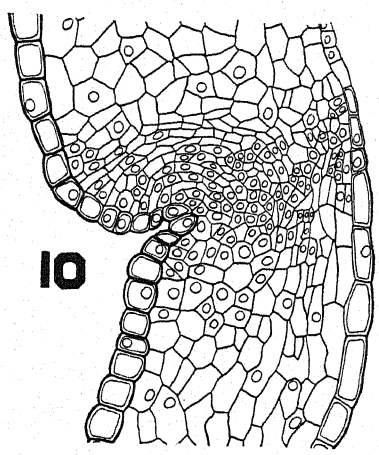
As the fruit matures, changes become evident in the cells making up the wall of the ovary. Only a few meristematic cells remain in the wall and these cells are found in the region of future dehiscence. The outer epidermis is composed of thick walled cells. In the base a layer of cells next to the outer epidermis is also thick walled (fig. 15). The inner epidermis of the lid and the adjacent layer of cells are thick walled, but this lignification does not proceed completely down to the zone of dehiscence.

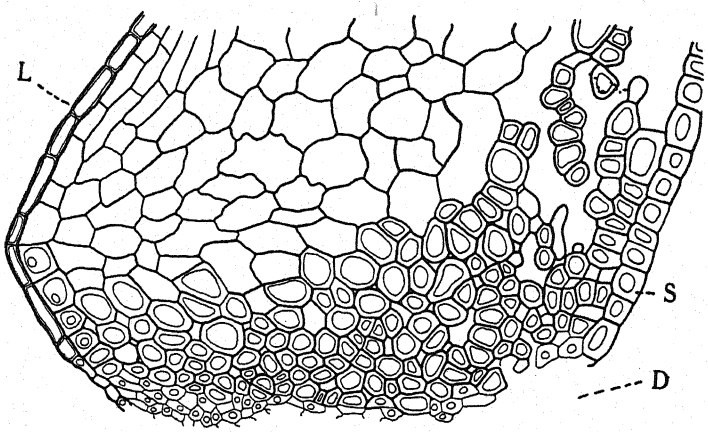
Fig. 1–6.—Fig. 1. *Portulaca grandiflora,* longitudinal section through ovary of young flower showing half-inferior ovary (O) with hypanthium (H), ×25.—Fig. 2. *P. grandiflora,* longitudinal section of right side of ovary showing an increase in prominence of ridge (R), beginning of lignification of epidermis of future lid and spongy (SL) and compact layers (CL) of wall of ovary, ×528.—Fig. 3. *P. grandiflora,* longitudinal section of right side of wall of ovary showing the breakdown of cells in the spongy layer (SL), ×172.—Fig. 4. *P. grandiflora,* longitudinal section of the right side of wall of ovary showing dehiscence almost completed, ×352.—Fig. 5. *P. grandiflora,* longitudinal section of right side of wall of ovary showing dehiscence completed. Lid (L) is free from base (B) which has abundant sclerenchyma (S). The ridge (R) was produced when stamens and perianth dropped, ×90.—Fig. 6. *P. grandiflora,* oblique section of base of fruit showing extent of sclerenchyma (S), ×90.

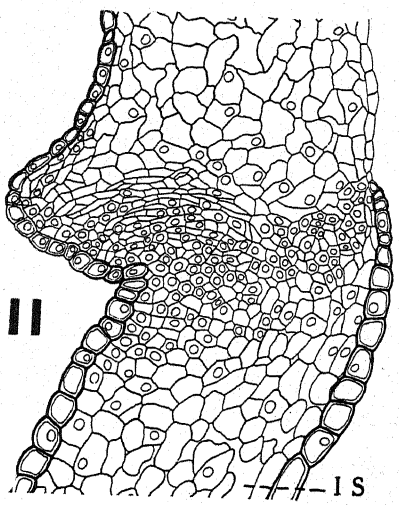

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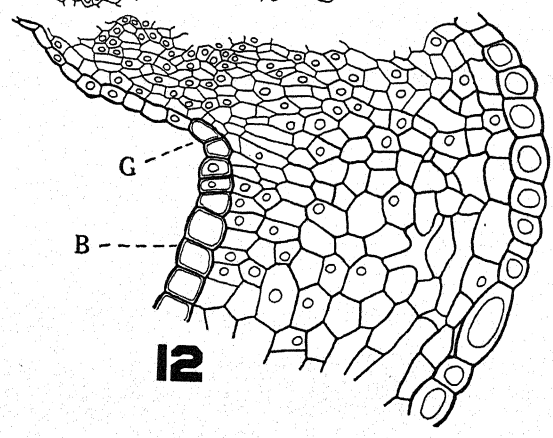

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The separation of the lid from the base involves the rupture of cell walls as well as the pulling apart of adjacent cell walls.

HISTOLOGY OF PLANTAGO PUSILLA.—In this species the future zone of dehiscence consists of a more extensive group of meristematic cells (fig. 16 and 17) than in *P. major*. The outer epidermal cells with the exception of those near the zone of dehiscence are heavily walled. The layer of cells next to the outer epidermis is composed of the largest cells seen in the wall of the ovary. A groove has been produced between the future lid and the future base. The inner and the outer epidermal layers across this groove are meristematic in appearance.

At the time of dehiscence (fig. 18) the cells of the outer epidermis of the lid just above the rupture are elongated transversely. The meristematic region is no longer apparent although the cells here are comparatively thin walled and smaller than the adjacent cells. The layer of cells next to the outer epidermis has increased in size and has thick walls except in the zone of dehiscence. It is apparent that the dehiscence as far as completed (fig. 18) involves the rupture of walls as well as the separation of the cells.

HISTOLOGY OF PLANTAGO MARITIMA.—It is probable that in this species as in *P. major* the young wall of the ovary is also composed of four meristematic layers of cells. In older stages the region of future dehiscence is very evident (fig. 19) in comparison with the two related species. The outstanding feature in this species is the formation of a groove on the inner wall of the ovary as well as on the outer. The cells across the wall of the ovary between these two grooves are meristematic. The outer epidermis becomes thick walled except at the zone of dehiscence.

When dehiscence is completed (fig. 20), a few meristematic cells can be seen on the upper margin of the base with an indication of rupture of a cell wall. The inner epidermis of the lid, the layer of cells next to the inner epidermis, and the cells above the rupture connecting the inner with the outer epidermis are heavily lignified.

DISCUSSION.—Dehiscence in the pyxidium cannot be accounted for completely by differential drying. It occurs while both halves of the fruit still contain living cells, and there is not the shearing action which would be apparent if one part were contracting more than the other. One would not be justified in saying that special lines of dehiscence produced the circumscissile split since all series examined show that the split is along no specific line and could not be definitely predicted.

In *Portulaca grandiflora* as the time for dehiscence nears, the differentiation of sclerenchymatous tissue in the base of the capsule makes this structure very rigid, being especially abundant in the ridge (fig. 5) left when the stamens and the perianth dropped. No sclerenchyma except outer epidermis is produced in the lid. The epidermal cells in the region of dehiscence however are comparatively small and constitute a zone of weakness.

The breakdown of cells in the spongy layer of the wall of the ovary greatly reduces the number of cells concerned in dehiscence. The inner epidermis retains its continuity, later coming in contact with the compact layer of the wall of the ovary. It seems only logical to conclude that the ripening seeds supply the force that accounts for the taking up of this new position.

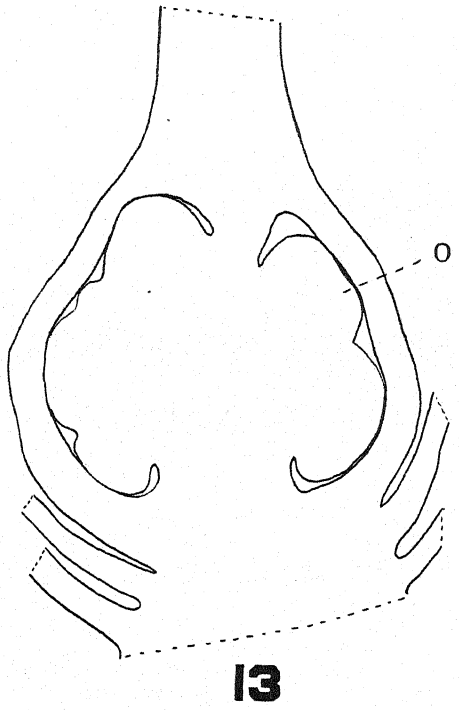
Since the long axis of cells in the zone of dehiscence is in the direction of rupture, it is easier for the cells to separate there; or if the walls are broken, the number of walls concerned is considerably reduced.

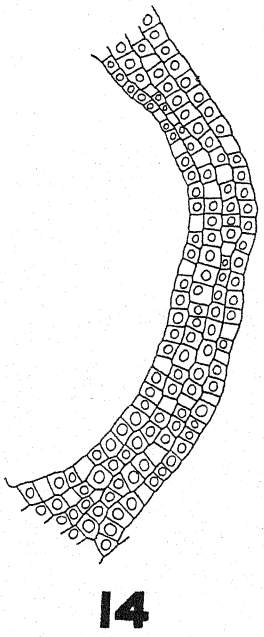
It is evident that the lid is moved upward in dehiscence and is not sheared off sidewise. The lifting force must be provided by the ripening of a large number of seeds or at least by the fact that these seeds do not decrease in size as the capsule shrinks upon drying.

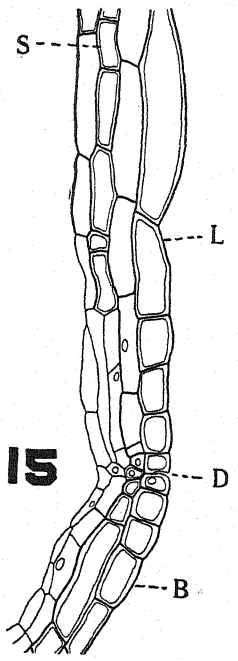
Dehiscence in *Hyoscyamus niger* is even more startling and clear-cut than in *Portulaca grandiflora*. The lid is very thick and solid in comparison with the base. The inner and the outer epidermis of the lid become heavily lignified, the inner more so. In addition to these lignified epidermal layers, there is just above the separation an extensively lignified area extending from the outer to the inner epidermis. Other lignified cells are formed in an irregular zone next to the inner epidermis of the lid; between these are numerous and large intercellular spaces.

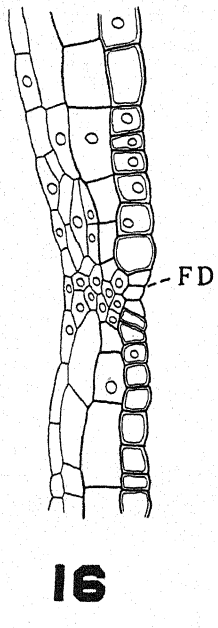
Lignification is not so extensive in the base as in the lid. In the former only epidermal layers appear to be concerned. The outer and the inner epidermis in the zone of dehiscence remain comparatively small in size and unlignified. As a result there is a zone of weakness which is still weaker on account of the meristematic cells between these two points. The rupture does not occur at the thinnest portion of the wall of the ovary, but it occurs at the place of least mechanical resistance. Differential drying

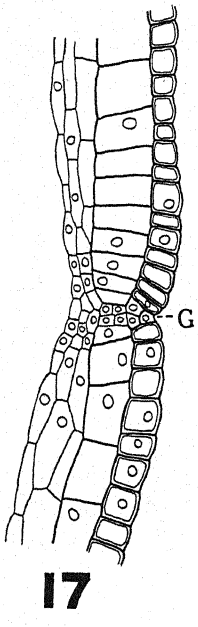
Fig. 7–12.—Fig. 7. *Hyoscyamus niger*, habit of fruit (after clearing in xylol) showing distinct lid (L) and base (B). Dehiscence will occur in the ridge (R), ×8.—Fig. 8. *H. niger*, longitudinal section of young ovary showing placental lobes, ×34.—Fig. 9. *H. niger*, longitudinal section of left side of wall of ovary in future zone of dehiscence showing ridges (R) with shallow groove (G) between, ×178.—Fig. 10. *H. niger*, longitudinal section of left side of wall of ovary showing thickened walls of epidermal cells and extent of meristematic area in future zone of dehiscence. The outer epidermis is on the left, ×190.—Fig. 11. *H. niger*, longitudinal section of left side of wall of ovary showing differentiation of epidermal cells in future lid and base. Intercellular spaces (IS) are numerous in the base. The outer epidermis is on the left, ×178.—Fig. 12. *H. niger*, longitudinal section of left side of wall of ovary showing dehiscence (D) completed. The lid (L) has much more sclerenchyma (S) than the base (B). The dehiscence does not pass through the formerly prominent groove (G). The outer epidermis is on the left, ×190.

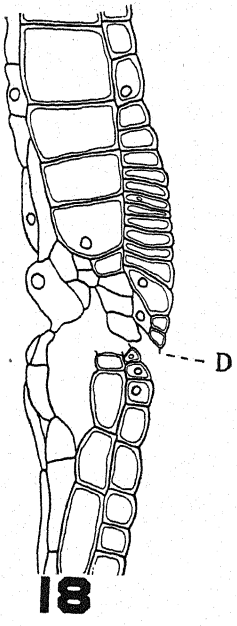


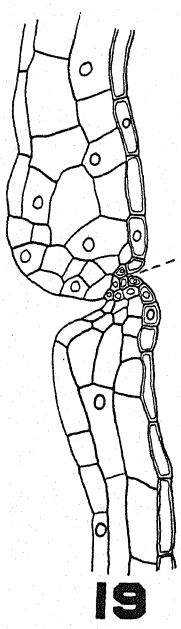












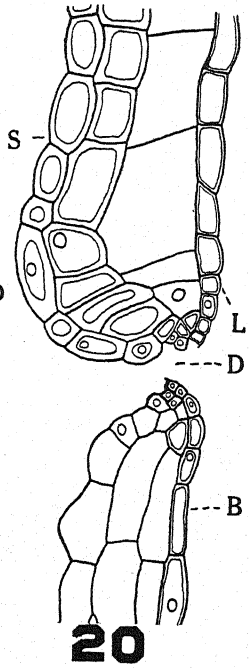


Fig. 13–20.—Fig. 13. *Plantago major,* longitudinal section of young ovary bearing several ovules (O), ×178.—Fig. 14. *P. major,* longitudinal section of right side of wall of young ovary showing 4 layers of cells, ×303.—Fig. 15. *P. major,* longitudinal section of right side of wall of ovary showing probable zone of dehiscence (D) separating lid (L) and base (B). Lignified cells (S) are more abundant in the lid than in the base, ×303.—Fig. 16. *P. pusilla,* longitudinal section of right side of wall of ovary showing future zone of dehiscence (FD) and significant features of outer epidermis, ×303.—Fig. 17. *P. pusilla,* longitudinal section of right side of wall of ovary showing a definite groove (G) opposite the future zone of dehiscence, ×303.—Fig. 18. *P. pusilla,* longitudinal section of right side of wall of ovary showing dehiscence (D) almost completed, ×303.—Fig. 19. *P. maritima,* longitudinal section of right side of wall of ovary showing future zone of dehiscence (FD), ×303.—Fig. 20. *P. maritima,* longitudinal section of right side of wall of ovary showing dehiscence (D) completed. Sclerenchyma (S) is more abundant in the lid (L) than in the base (B), ×303.

does not seem to have had any effect on the process since even the meristematic cells (at least those not involved in the rupture) are still normal in all characteristics. The dehiscence has been accomplished by the rupture of walls of cells as well as by their separation.

Dehiscence is not shown so clearly in *Plantago major* as in the preceding and the following species, but the writer feels that an older fruit may show slightly different results. However, a zone of mechanical weakness has been definitely formed even in the stages examined. The lid and the base contain enough sclerenchyma to make them firm; between them is an area which is largely meristematic. The outer epidermis throughout the region of dehiscence has thickened walls; but these epidermal cells are not quite so large as the thick walled epidermal cells covering the lid and the base. The outer epidermal cells concerned with the dehiscence have almost entirely pulled apart, and it seems reasonable to conclude that rupture of the walls of the cells and separation of the cells will complete the phenomenon.

In *Plantago pusilla* are seen many repetitions of significant factors in dehiscence. In this species a distinct external groove forms at the zone of rupture. The outer epidermal cells here become somewhat thick walled, but they are smaller than the outer epidermal cells of the lid or of the base; consequently this is a weak place in the wall of the ovary. The tenacity of cells in the zone of dehiscence is further reduced since the layer next to the outer epidermis in both the lid and the base is thick walled except in the future splitting region. In the upper portion of the lid, the inner epidermis and the adjacent layer of cells are lignified further adding to the rigidity of the lid. The line of dehiscence involves the rupture of walls of cells and the separation of cells, forming an indefinite break.

Of the species of *Plantago* examined, *P. maritima* presents the most specialized and obvious method of opening of the capsule. The lid in this species is thicker than the base and is firm on account of the large and lignified cells of the two innermost layers. The region of meristem is definitely persistent and involves even the outer epidermis. The pronounced inner groove of the wall of the ovary is due to the much greater size of cells immediately above the zone of dehiscence and the fewer cells concerned in the zone of dehiscence. Dehiscence includes both the separation of cells and the rupture of walls of cells, with the former predominant as in the preceding species.

These conclusions are somewhat in accord with Guppy (1912) who states, "Regarding the difference between the two processes from merely a physical standpoint, we can say that whilst the capsule dehisces and dries, the legume dries and dehisces. By removing a single letter we can at the same time express a biological distinction by saying that whilst the capsule dehisces and dies, the legume dies and dehisces." Therefore, in the case of the capsule the mechanism of dehiscence has to do with a living fruit, whereas in the legume a dead fruit is concerned.

SUMMARY

A number of previous investigations explain dehiscence by tensions in the fruit which are set up by differential drying. While that may be true for the pod and the follicle, the present investigation leaves it doubtfully true for the pyxidium. The lid and the base of the mature pyxidium in all cases studied is more or less rigid due to the production of sclerenchyma. A zone of mechanical weakness is formed between the lid and the base due to alignment of the cells in this zone, size of the cells, number of cells concerned, thickness of walls of cells, meristematic state of the cells, or various combinations of these reasons. It appears that the splitting force is due to the development of seeds that completely fill the cavity of the ovary and maintain their mature size while the wall of the fruit shrinks upon drying.

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CONCERNING THE DUALITY OF THE MITOCHONDRIA AND THE VALIDITY OF THE OSMIOPHILIC PLATELETS IN PLANTS [1]

Earl H. Newcomer

THE PRESENT study is largely concerned with the validity of Guilliermond's and Bowen's theories and evidence of the dual nature of mitochondria in fixed material of plants, and, since technically related, with those bodies in the cytoplasm called osmiophilic platelets by Bowen. The problem of cytological technics relative to the demonstration of mitochondria is also considered and some suggestions are proffered for a simpler and more rational terminology in reference to mitochondria.

In 1920 and in many subsequent papers, Guilliermond and his associates (1920, 1932, 1933, 1941) described two categories of elements among mitochondria in plants which he designated as the active and inactive chondriome. The former eloborated starch and became plastids, whereas the latter retained their original morphology and remained inactive. These inactive mitochondria in flowering plants were considered homologous with those of the fungi, which in turn he homologized with the mitochondria of animal cells. Of the mitochondria in flowering plants, he said (1941, p. 96), "In most cases it is absolutely impossible to distinguish in meristematic cells, which are destined to become plasts and which remain inactive. . . . They offer (p. 113) moreover, and this is much more important, the same histophysical characteristics (same refractivity, same viscosity, same process of alteration and the same histochemical characteristics, even to their behavior with a great number of chemical reagents and dyes). . . . The plastids, therefore, are distinguished from the other chondriosomes only by the fact that they are the centers of very active elaborations which considerably modify their shape. This is true in cells lacking in chlorophyll in which plastids elaborate starch, and especially true in green cells in which the plastids become voluminous because of the chlorophyll which they accumulate. Moreover, these modifications of forms may be only transitory. The amyloplasts in cells without chlorophyll, as soon as the starch has been absorbed, go back to their forms of typical chondriosomes. The chloroplasts themselves may in some cases lose their chlorophyll and return to their original state as chondriosomes. In a word, the chondriosomal form is the form taken by these organelles during the functionally inactive period. The only distinction, therefore, that exists between the animal and fungal cell on the one hand, and the cell of the green plant on the other, is the presence of plastids, the second category of chondriosomal elements. This distinction is related to the existence of the chlorophyll function which characterizes green plants."

"These facts (pp. 113, 114), now exactly demonstrated by our work and that of our fellow students, carried on over a period of thirty years, have led us to formulate the theory of the duality of the chondriome in chlorophyll-containing plants. This consists in stating that the chondriosomes of chlorophyll-containing plants are composed of two categories, having between them the same relationships which the heterochromosomes bear to the autochromosomes, the first category (the *genuine chondriosomes*) being very similar to the chondriosomes of animals and fungi, the second category (the plastids) composed of a supplementary line of chondriosomes related to photosynthesis, which characterizes these plants. It is obvious that the two categories must, after all, possess differences in chemical constitution. Otherwise it could not be explained why one has functions which the other does not have. . . . The question is, therefore, definitely solved as far as the facts are concerned and it is demonstrated that the chondriosomes and plastids are two individual cellular components with the same lipoprotein constitution, capable of presenting identical shapes but developing side by side without any genetic bond between them."

Another theory of the duality of mitochondria in plants was presented by Bowen (1919, 1926, 1927, 1928, 1929a, 1929b, 1929c), who was also admittedly motivated by the assumed logical necessity of finding homologous structures in plant and animal cells. He said (1928), "if the protoplasm of plants and animals is 'essentially identical,' we should expect to find in the plant cell materials which, in general structure and function, are homologous to those enumerated in the animal cell." Bowen described and defined five cytoplasmic components of plant cells: plastidome, pseudochondriome, osmiophilic platelets, vacuome, and oil droplets. The plastidome and pseudochondriome, which correspond with Guilliermond's active and inactive chondriome respectively, are, according to Bowen, functionally distinct and differ in staining behavior, morphology, structure and behavior during cell division. In the plastidome, the constituents of which he called the proplastids or archiplasts, staining with the vital dyes is noticeably slower than in the pseudochondriome. Polymorphism is a property of the plastidome and the proplastids are never granular in structure, in contrast to the pseudochondriome. The peculiar radial orientation of the plastidome at the polar caps during mitosis and the absence of pseudochondriome in root-cap cells constituted additional criteria for their functional differentiation.

Their distinction was most clearly shown in the *Thallophyta, Bryophyta,* and *Pteridophyta,* in which Bowen claimed to have demonstrated the permanence in shape and numbers of plastids in all phases of the life cycle but found associated with them small spherical bodies which constituted what he called the pseudochondriome. The plastidome in

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flowering plants, according to Bowen, has lost its clear-cut individuality because of its failure to maintain a chlorophyll condition throughout the life cycle. The pseudochondriome, on the other hand, has retained its permanence of shape from unicellular organisms throughout the vicissitudes of plant evolution and constitutes the homologue of the mitochondria in animal cells.

The osmiophilic platelets were first described by Bowen in the cytoplasm of plant cells with the aid of osmium tetroxide, largely by the Kolatchev technic, as follows (1928): "In morphology, the osmiophilic platelets are characterized by a ring or a disc shape, the periphery blackening very intensely and rather selectively with osmic acid. The result is the appearance of a black ring when seen in plane view, a simple, straight, sometimes slightly curved rod when in profile, and ellipses of various shapes when seen in intermediate aspects. . . . These optical appearances seem to demonstrate that an osmiophilic platelet is not a spherical vesicle or vacuolar structure of any kind, but a flattened body which, when seen on edge, could alone give the appearance of a rod. . . . The intensity of blackening is also subject to great variation, depending chiefly upon the length of osmication, and the intensity and frequency of artefacts both increase rapidly after the optimum osmication-time has passed. . . . We have to deal, in other words, with a cytoplasmic constituent of the same rank as the chondriosomes, Golgi apparatus, plastids, etc. The osmiophilic platelets are to be viewed as one of the generalized constituents of plant cytoplasm." He homologized them with the Golgi apparatus of insect cells and the sperm acrosome in the mosses.

For the purpose of defining terms and reducing to synonomy the many terms occurring in the literature in reference to mitochondria, it is proposed that the term mitochondria (*mitos,* thread—*chondrion,* small grain), sing. mitochondrion, coined by Benda in 1897, be retained on the grounds of etymology, priority, common usage and adequacy for our present knowledge of the subject. The terms mitosome and chondriosome should logically be used to refer to the thread-or rod-shaped and granular mitochondria, respectively, and the term mitochondriome to refer, in a collective sense, to all the mitochondria in a cell or tissue. Since many of the synonyms for mitochondria arose out of polemical or other equally irrelevant considerations, it is believed that the adoption of these simplifications in terminology will aid in the mutual understanding of workers in this field and that it might assist in removing the problem from the domain of the hardy few.

The terms proplastid and archiplast are generally accepted as referring to those elements of mitochondrial morphology which show evidence of starch or other secretion.

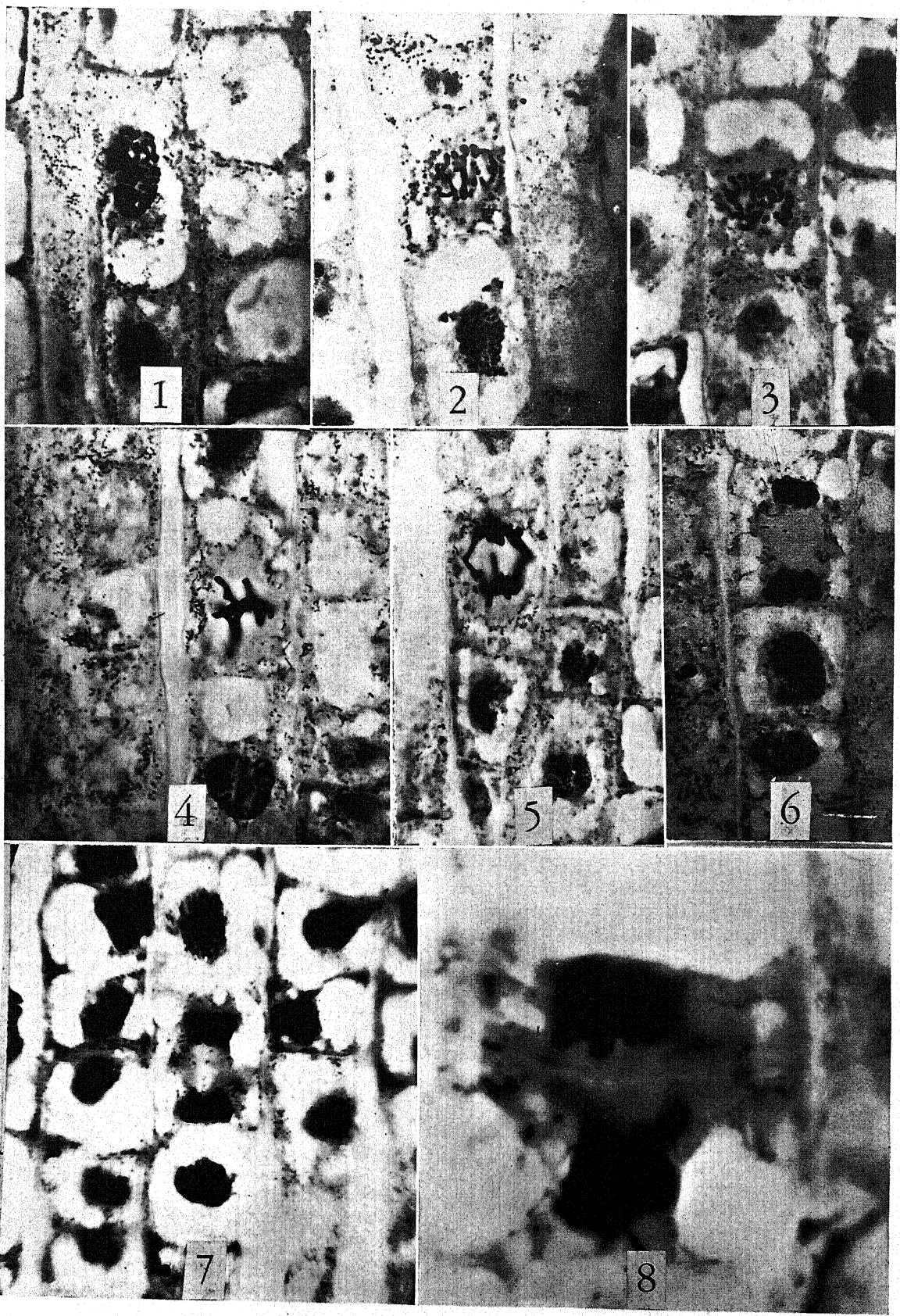
MATERIALS AND METHODS.—A variety of plants and tissues was tentatively chosen, some on the basis of precedent for the purpose of comparison, others in the hope of finding new, more favorable materials. To avoid useless descriptive duplication, but to preserve some latitude for comparative purposes, those retained for this report consist of the root tips of *Narcissus, Pisum,* and *Zea,* the germinating seeds of *Zea* and *Triticum,* petals of *Hyacinthus,* shoot tips of *Elodea,* somatic and reproductive tissue of *Nicotiana* and *Ginkgo,* pollen tubes of *Begonia,* and the ovuliferous tissue of *Iris.*

Considerable emphasis in the present study was placed upon technic because it is believed that therein lies the explanation for the disparate results of the many investigators and that it constitutes the real obstacle to the further elucidation of the problem of mitochondria. For this reason, every killing fluid and mitochondrial technic found in the literature was used as well as many modifications and original concoctions. A total of one hundred and eighty-nine such technics was used. In addition, materials killed and dehydrated by the modified Altmann freezing-drying technic (Goodspeed and Uber, 1934), were made available by the kindness of Professors T. H. Goodspeed and Fred M. Uber. This material was infiltrated with paraffin oil and low melting-point paraffin, sectioned at 5 μ and subsequently stained or subjected to microchemical reagents.

In staining fixed and sectioned materials, every mitochondrial stain and technic found in the literature was used but aside from the possible esthetic pleasure of viewing a multi-colored preparation achieved at considerable time and effort, the best and most consistent results were secured with the use of Haidenhain's iron-alum haematoxylin without a counterstain after bleaching with potassium permanganate and oxalic acid.

Another method of coloring mitochondria which sometimes produced excellent results but the capriciousness of which was so great that it was finally discarded as untrustworthy, was a modification of the Kolatchev technic in which Zirkle's fluid was substituted for Champy's (Newcomer, 1940a). In the publication just referred to there is an unfortunate mistake in the formula for Zirkle's fluid. The salts are all printed as three per cent solutions, whereas they should be dissolved directly by the weight stated into the indicated quantity of water. The mistake was not a typographical error but an oversight of my own.

Zirkle's fluid (Zirkle, 1929) produced the best fixation for mitochondria of all the formulas found in the literature. The addition of one to two drops of ammonium hydroxide to 100 c.c. of the fluid usually improved the fixation image and the concentration of the fluid may be cut to one-half for the more delicate tissues. A potentiometer was not available save for the last part of this study, but from Zirkle's work (1928) and my own corroborative observations, it seems clear that the pH of the killing fluid is one of the most important factors involved in the fixation of the cytoplasm with the salts of chromium. This factor may be responsible


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for some of the confusion and disparate results among the investigators of mitochondria.

Zirkle's fluid at its best, however, produces a basic image, preserving the mitochondria, hyaloplasm and nuclear lymph (fig. 15, 17, 32, 33), but it does not preserve the chromatin nor dividing chromosomes adequately. For this reason a search was made for other chemicals which might produce a more complete fixation, i.e., would preserve both cytoplasmic and nuclear constituents of the cell so that the behavior and distribution of the mitochondria could be observed during mitosis.

Baker (1932) suggested the use of parabenzoquinone dissolved in isotonic saline solution as a good pre-treatment for the preservation of mitochondria in animal cells. He later (1932a) retracted the statement that parabenzoquinone was a powerful fixative for mitochondria and thought the effect of the omission of fat solvents from mitochondrial fixatives was exaggerated because he observed them after Bouin's fixation and quoted Romeis as having seen them after Carnoy fixation. My own limited experience and experiments with the use of the quinones (fig. 1–9) indicate that when used alone as a preliminary treatment to be followed by Zirkle's fluid or mixed with Zirkle's fluid, they have yielded some excellent results. The technic is not yet standardized but a potentiometric study is under way using quinone, hydroquinone, and quinhydrone in the hope that it can be standardized. The fixation can be described as complete, *i.e.*, the chromosome morphology and structure are beautifully preserved as well as the chromatin in interphase stages (fig. 1–6). In addition, the cytoplasmic constituents are well preserved and can be stained readily. The coiled spirals of chromosome structure are shown in as good or better detail than the standard chromosome fixatives render them. An interesting observation, irrelevant to the subject of mitochondria but relevant to the subject of fixation is the image of the cell plate presented by a quinone-Zirkle fixation. The cell plate appears (fig. 8), as a vacuolated line across the phragmaplast instead of the usual solid line presented by other fixatives. Whether this is an artefact or a truer than usual picture of cell wall formation is not known. In view, however, of the apparent fidelity of the general picture produced by this fixation, the figure may have some validity.

A number of microchemical methods were used for qualitative purposes but since none of the methods were original nor the results other than corroborative, they will be briefly mentioned later in this report.

Illustrations were deliberately limited to photomicrographs and are untouched.

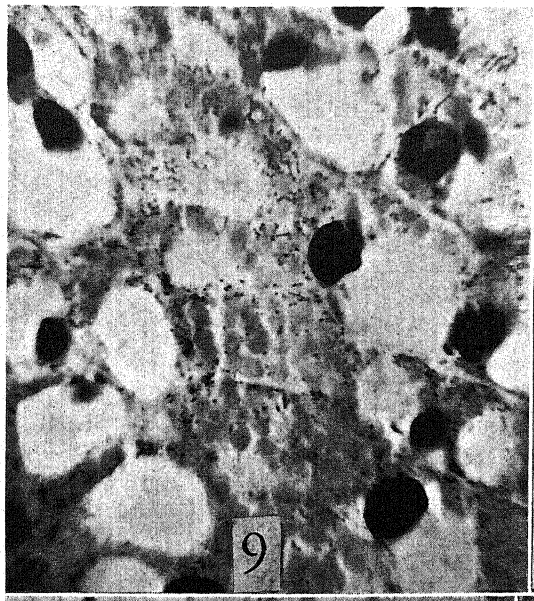
Observations on fixation.—The object of the present investigation was not a comparative study of the effects of the variously recommended mitochondrial technics upon the mitochondria of plants, but a study of the mitochondria themselves. The technical difficulties encountered, however, and the duplication of much of the previous investigators' work and a comparison of their results with my own, led to the extended experimental work on fixation. This was necessary because there is not as yet a single mitochondrial technic which will yield a consistent fixation image comparable with any of the numerous chromosome technics. The difficulty of fixation seems to be the primary obstacle to the further elucidation of the problem as well as having been responsible for much of the confusion thus far in the study of the cytoplasmic constituents of the cell. It also appears futile to continue investigations in this field with the technics available. The subject needs a fresh approach and above all, a technic which can be standardized and which will yield comparable results in the hands of any competent investigator.

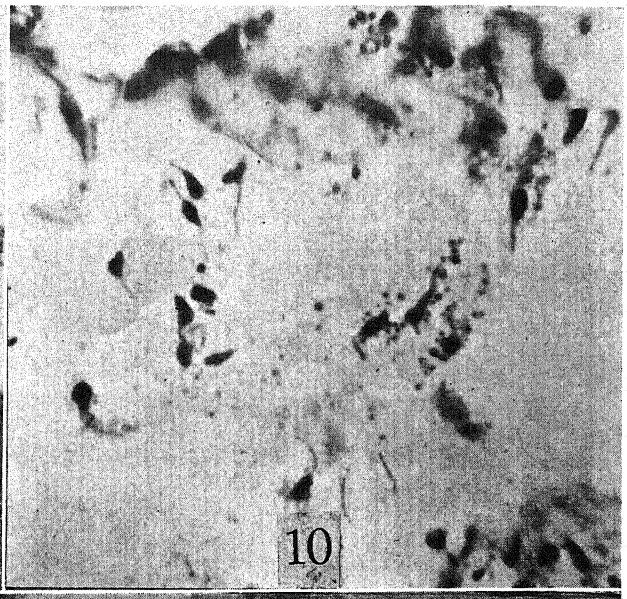
In the past, Regaud's fluid has been one of the most widely used for mitochondria, and Guilliermond based nearly all his conclusions upon its use. He stated that (1941) "The mitochondrial fixatives, *i.e.*, those of Benda, Meves, Regaud, and formaldehyde as well, preserve the chondriosomes, on the contrary, as faithfully as possible in the forms they show when alive." Of Regaud's technic, Bowen (1927) reported it worthless, N. H. Cowdry (1920) found it inadequate, and Weier (1931) said, "That I do not possess the complete sporogenetic history of the plastid after fixation with Regaud's fluid was due to the fact that this fluid does not preserve the plastid." The technics of Benda and Meves are even less adequate than that of Regaud in our hands for the preservation of the mitochondriome.

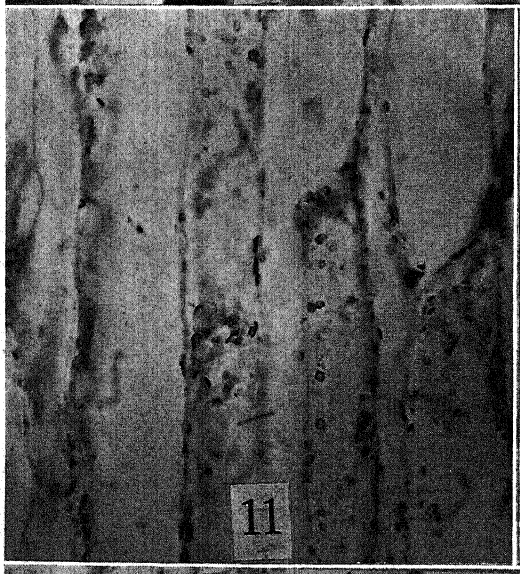
The emphasis of Bowen's interpretations was based upon the results obtained by the impregnation methods of Weigl, Mann-Kopsch, and especially Kolatchev. Upon the efficacy of the latter method rests the validity of the osmiophilic platelets. The reliability of the Kolatchev technic and methods of impregnation generally are open to question as will be shown later in this paper.

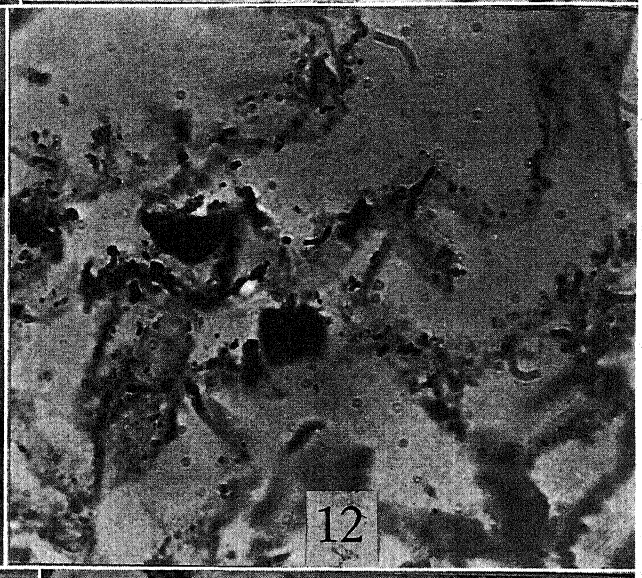
On the duality of the mitochondria in plants.—Guilliermond's theory of the duality of

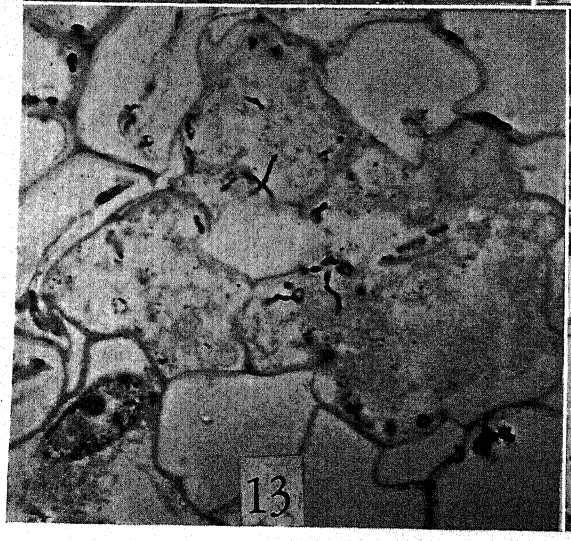
Fig. 1–8.—Fig. 1. Prophase chromosomes and mitochondria of root-tip of *Narcissus* pre-fixed in mixture of 0.5% quinone in 0.4% NaCl followed by Zirkle's fluid. ×900.—Fig. 2. Prophase chromosomes and mitochondria of root-tip of corn. Fixation as in figure 1. ×900.—Fig. 3. Root-tip of corn fixed with mixture of 10 c. c. of 0.5% quinone in 90 c. c. of Zirkle's fluid. ×900.—Fig. 4. Lateral view of metaphase of *Narcissus* root-tip fixed as in figure 1, showing typical orientation of mitochondria. ×900.—Fig. 5. Late anaphase of *Narcissus* root-tip, fixed as in figure 1. ×900.—Fig. 6. Telophase of *Narcissus* root-tip, showing mitochondria free of spindle area. Fixation as in figure 1. ×900.—Fig. 7. Late telophase of *Narcissus* root-tip fixed in mixture of quinone and Zirkle's fluid as figure 3. ×900. —Fig. 8. Telophase figure from *Narcissus* root-tip showing vacuolated appearance of the cell plate. Fixation as in figure 3. ×3000.

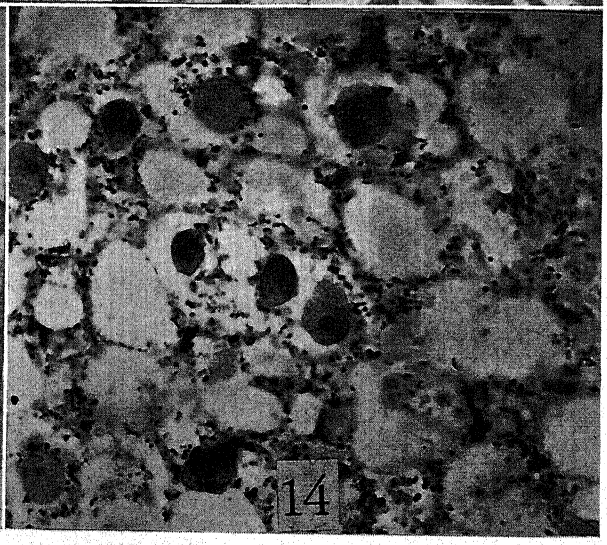

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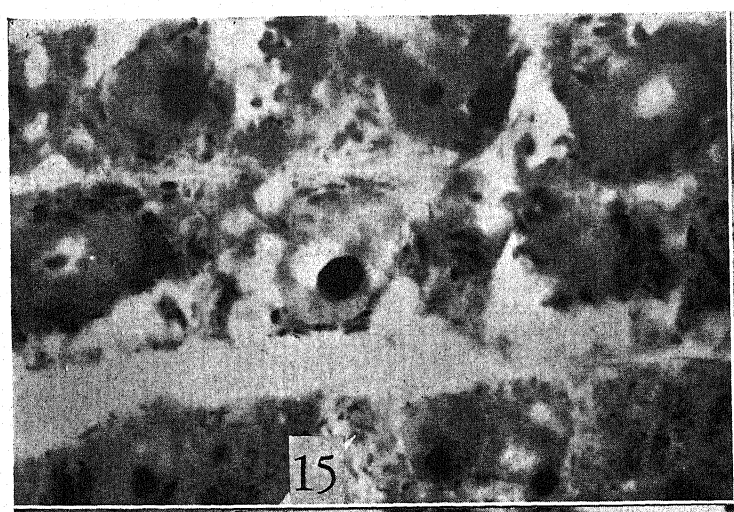

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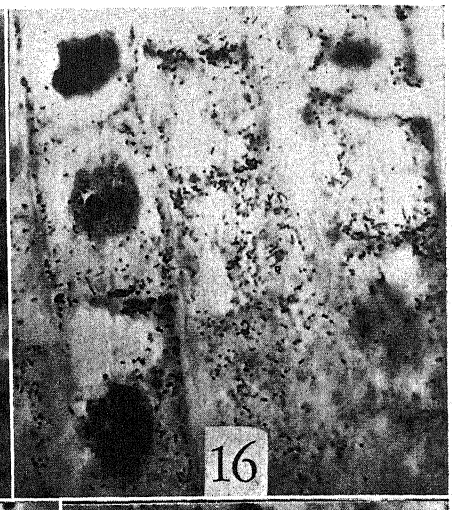
the mitochondria in plants rests upon *a priori* considerations having to do with concepts of homology. Since plants possess both mitochondria and plastids, the latter arising from the former, and since animal cells contain mitochondria similar in all respects to those of plant cells but do not contain plastids, it is logical to assume, according to Guilliermond, that the mitochondria in plant cells must be of two types, one kind the homologue of animal mitochondria, *i.e.,* the inactive or genuine chondriosomes, the other, which gives rise to plastids and though mitochondrial in chemistry and morphology, constitute another category, the active mitochondria. Despite the fact that no chemical or morphological distinction has ever been made between them, he insists that, "It is obvious that the two categories must, after all, possess differences in chemical constitution. Otherwise it could not be explained why one has functions which the other does not have." There is no cytological evidence to support such a separation of mitochondria in plants into two categories. Considerable emphasis for such a separation is laid upon the evidence from *Elodea* of which he said (1941), "It has been seen that among the elements which constitute the chondriome in meristematic cells of the bud of *Elodea canadensis,* it is possible to distinguish between the chondrioconts which become chloroplasts during cellular differentiation, and the granular mitochondria which do not participate in this phenomenon but elongate into rods and later into chondrioconts." I assume this to mean that the chondriosomes do elongate to form mitosomes but that the development of these mitosomes ceases there and that the mitosomes which form plastids are identical in morphology to the mitosomes originating from chondriosomes but that they are genetically distinct and of a chemically different constitution. Such a distinction would appear very difficult to observe in a living cell or one fixed with Regaud's fluid. In any event, the implication that the chondriosomes do not give rise to plastids in *Elodea* is not supported by the results from this study (fig. 15). The origin of plastids from both mitosomes and chondriosomes has been the most consistent observation in the present study (fig. 10–20) and similarly, in the few cases of apparent plastid reversion observed, the ultimate reversion forms are both granular and rod-like in morphology (fig. 13, 30, 31, 38, 40).

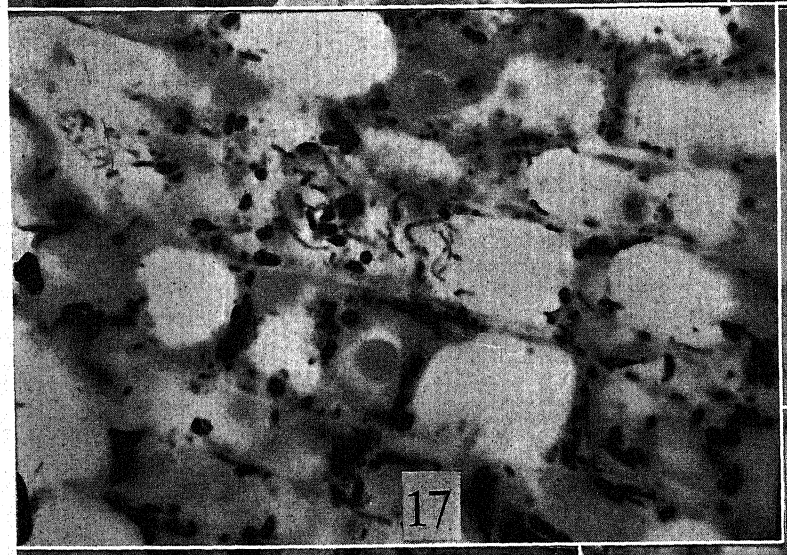
As for the functional distinction between the mitochondria of plant and animal cells (*viz.,* the plastid-forming ability of the former), this is no more profoundly marked than the successive transformations in function, chemistry, and morphology of a mitochondrion into leucoplast, chloroplast, and chromoplast as observed in the tomato or squash plant. Furthermore, as opposed to the above single instance of functional differentiation between the mitochondria of plants and animals which Guilliermond considers insurmountable, what of the numerous functions ascribed to mitochondria in both plants and animals? For example, some mitochondria in plants become oleoplasts (Faull, 1935) and secrete oils and fats; others develop into leucoplasts and can transform hexose sugars into starch, whereas in many monocots the plastids have either lost or never had the ability of starch synthesis. In animal cells similarly the mitochondria in various tissues have been identified with many diverse secretory activities. Logically, therefore, according to Guilliermond's theory, each mitochondriome performing different functions should possess different chemical constitutions. This is not only *prima facie* improbable, but is totally without any experimental evidence. Functional distinction, therefore, does not seem to be a valid presupposition for attempting to separate the mitochondria of plants into two categories.

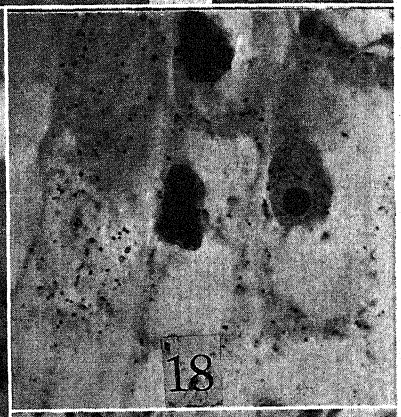
Genetic evidence would also seem to negate the validity or necessity of such a theory. The metabolism and, to a considerable extent, the morphology and behavior of the cellular constituents of an organism are conditioned by its genetic constitution. Thus, in corn, there are at least a hundred known instances of genic factors modifying the chlorophyll condition of the plastids from albinism to various degrees of virescence. The morphology of the plastids is similarly altered throughout the gamut of their possible changes (DuBuy and Woods, 1943; Woods and DuBuy, 1943). The fact that the fungi contain mitochondria but not plastids is no reason for homologizing the mitochondria of the fungal cell with those of the animal cell. Some of the higher seed plants are also fungal in metabolism, *viz.,* the orchids *Hexalectris, Corallorrhiza,* and *Tuphora;* members of genera of the *Ericaceae* such as *Monotropa, Monotropsis;* and *Obolaria* of the *Gentianaceae,* all of which are saprophytes. Other seed plants exhibit partial parasitism such as *Cuscuta, Buckleya,* and *Darbya,* and complete parasitism is exhibited by *Pyrularia pubera, Conopholis americana,* and *Epifagus virginiana.* Because these plants achieved their saprophytic or parasitic habits at an obviously later date than did the fungi, few would deny that the metabolic differentiation in both categories of plants was caused by the same thing, *viz.,* genetic change or mutation. It appears quite probable that the diver-

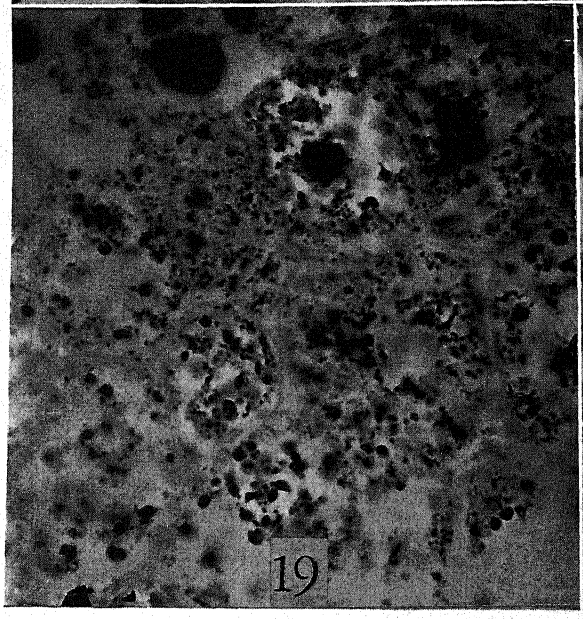
Fig. 9–14.—Fig. 9. Root-cap cells of *Narcissus* showing typical amyloplasts surrounded by mitosomes and chondriosomes. Fixation as in figure 3. ×900.—Fig. 10. Petal of Hyacinth showing transition of mitochondria into chromoplasts. Zirkle's fixation. ×1350.—Fig. 11. Corn root-tip back of meristem showing transitional stages of mitochondria to plastids. Fixation modified Kolatchev, bleached and no stain. ×900.—Fig. 12. Cells of the funiculus of the ovule of *Iris.* Zirkle's fixation. ×1350.—Fig. 13. Cells from scutellum of a two-day germinated corn seed showing amyloplast origin from mitochondria or the reversion of amyloplasts to mitochondria and division of mitochondria by fission. Fixation as in figure 1. ×900.—Fig. 14. Root-cap cells of corn fixed by modified Kolatchev technic. Bleached but not stained. ×900.

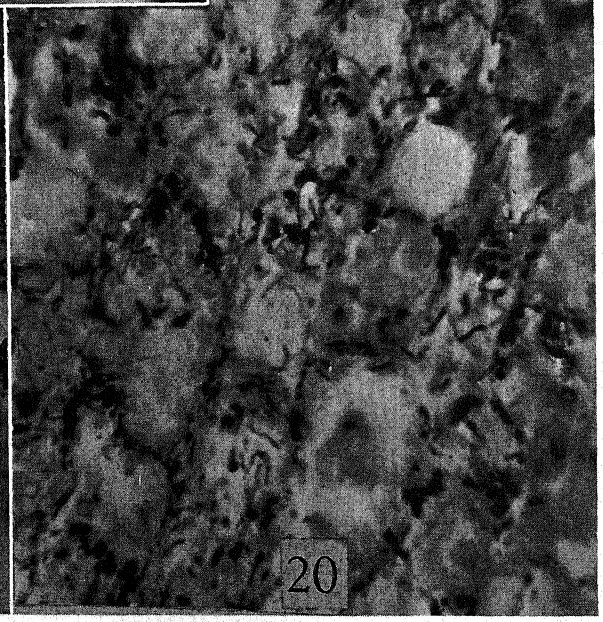

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gence of the plant and animal kingdoms from the primordial cells or organisms was similarly conditioned and that it is futile to press the concept of homology too closely. A simpler explanation, and one for which there is considerable evidence, would seem to suggest that the mitochondria of both plants and animals, while homologous structures, are functionally versatile in response to the genetic constitution and metabolic requirements of the organism.

If the recent research of Woods and DuBuy (1943) and DuBuy and Woods (1943) is corroborated suggesting the origin of plant viruses through mutation from constituents of the plant mitochondriome and the analogy between variegation-inducing plastids and cancer-inducing mitochondria, the present narrow conceptions of mitochondria will be found inadequate and a return to something like the eclectosome hypothesis of Regaud will doubtless be more appropriate, if indeed, Regaud's theory is not even now the closest approach to the facts of experimental evidence.

Bowen (1927, 1928) considered Guilliermond's theory "a matter of words," and Zirkle, whose researches upon the cytoplasmic constituents of plant cells (1928a, 1929, 1932, 1934, 1934a) were extensive, concluded that mitochondria and plastids are merely developmental stages of a single cell organ (1927, 1928). This conclusion was also supported by the work of Faull (1935) and Anderson (1936) on fixed and living material. Guilliermond's theory of the dual nature of the mitochondria in plants not only lacks plausability but has no experimental evidence for support. It might also be added that Guilliermond's positive attitude in conjunction with his numerous publications on the subject have created the illusion in the minds of many cytologists that the problem has been solved, and the net result has been one of stultification of further research on mitochondria in plants.

Bowen's claims for distinguishing two categories of elements in the mitochondriome of plants, while motivated also by an admitted preconception, are based upon more definite evidence, and a fuller comparison of experimental results can be made. According to Bowen (1929a) the plastidome (mitosomes) can be distinguished from the pseudochondriome (chondriosomes) by the radial orientation of the former at the poles during cell division and the greater precision of their distribution to the resulting daughter cells. The absence of transition stages of pseudochondriome into plastids, their vesicular structure and their random arrangement during mitosis serve to distinguish these bodies from the plastidome.

A comparison of figures 1–6 of this study with Bowen's figures (1929a–1929b) which similarly depict the behavior of mitochondria during mitosis, shows little agreement. Throughout the present study, no polar radial arrangement or precision of distribution of the mitosomes has been observed. An occasional radial arrangement of the longer mitosomes at the poles due to the presence of vacuoles has been observed but this does not occur in non-vacuolated cells. Segregation of the mitosomes and chondriosomes also appears to be a random process (fig. 4–7).

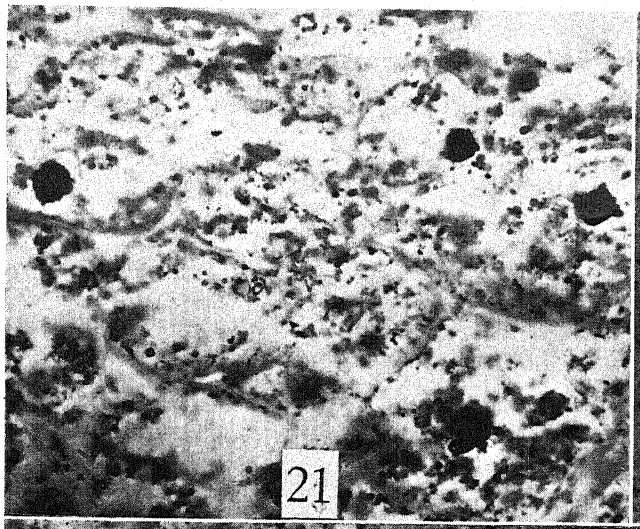
The vesicular structure which Bowen maintained was characteristic of the pseudochondriome was often observed in the mitosomes and proplastids (fig. 11, 13, 16, 21, 30). This variability of structure is especially common and varies with the technic used and it may represent the mitochondria in some phase of development or it may be an artefact. It is certain that it cannot be considered diagnostic.

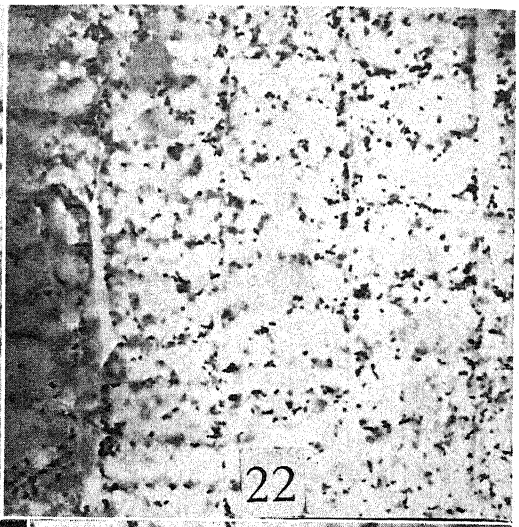
Early in Bowen's paper (1929a) on the plastidome and pseudochondriome, he made the following statement: "To summarize my conclusions on this much misunderstood matter, I have decided in the first place that there are two absolutely distinct categories of bodies in meristem cells—the proplastids and the pseudochondriomes. These bodies differ not only in morphology and functional history, but in their microchemical behavior. The fixation of the plant cell is so difficult that constant success with any method is not to be expected, more particularly with those which start with chrome-osmic fixation. When such fixatives really succeed, however, it is often possible to separate the plastidome and the pseudochondriome with diagrammatic clearness.

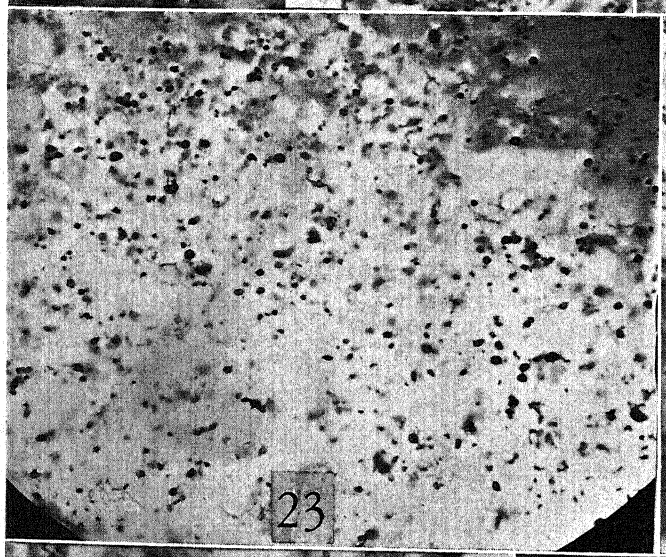
"But, after all, the important thing is not that these methods are variable and difficult—due to the inherent nature of plant material—but that their use permits the separation of two primary constituents of plant cytoplasm. Guilliermond's Regaud results are often unsatisfactory for the very reason that they do not permit the clear-cut differentiation of the two categories of bodies from each other. And this is a prime essential for the ultimate solution of the cytoplasm problem in plants."

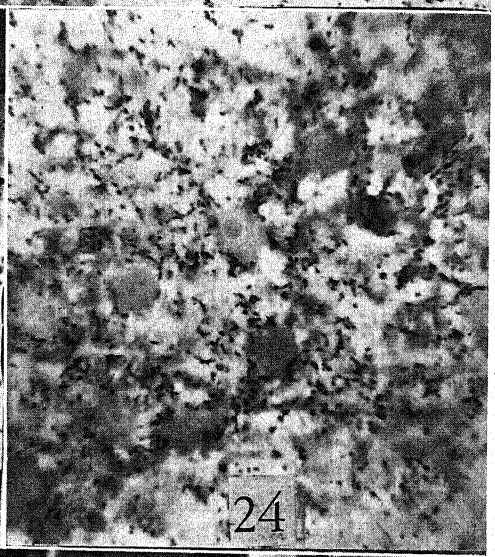
In the same paper, Bowen observed that, "It will be evident to any unbiased reader that the difficulties in which we now find ourselves in regard to the structural elements of plant cytoplasm are due primarily to a wrong approach to the whole problem. The cart has, in fact, been put before the horse.

Fig. 15–20.—Fig. 15. Longitudinal section of shoot-tip of *Elodea*. Cells on upper side of figure from the tunica, those on lower side from corpus. In tunica can be seen transition stages of both mitosomes and chondriosomes into chloroplasts. Zirkle's fixation. ×1350.—Fig. 16. *Narcissus* root-tip showing typical polymorphism of the mitochondria. Fixation as in figure 1. ×900.—Fig. 17. Root-tip of *Pisum*, fixed with Zirkle's fluid bleached and stained with haematoxylin. cf. with figure 20. ×1350.—Fig. 18. *Narcissus* root-tip showing transition of granular mitochondria (chondriosomes) into plastids. Fixation as in figure 1. ×900.—Fig. 19. Root-tip of corn fixed in quinone-Zirkle mixture showing plastid formation from a predominantly mitosome origin. ×900.—Fig. 20. Root-tip of *Pisum*, fixed by modified Kolatchev technic, showing typical polymorphism of mitochondria and proplastids. Bleached only, no stain. cf. with figure 17.

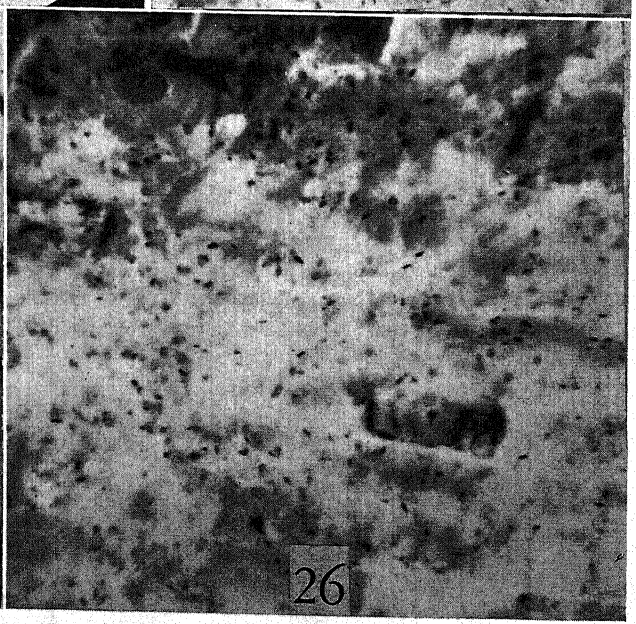

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Instead of studying the meristematic or embryonic plant cell for its own sake, almost without exception the problem has been approached from some theoretical preconception of chondriosomes or plastids."

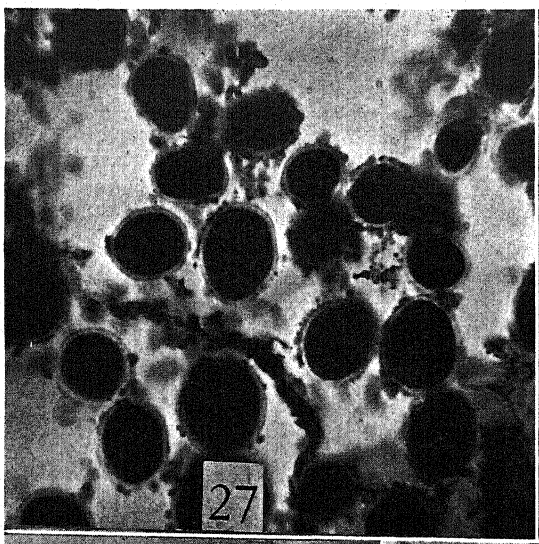
The unbiased reader may well agree that the study of mitochondria in plants has been retarded by theoretical preconceptions but Bowen's interpretations can hardly be called free from *a priori* concepts. The use of a killing fluid and stains which permit the clear-cut differentiation of two categories of bodies in the mitochondriome would seem, while convenient, obviously suspect, unless it could be proven that the fluid used had the exclusive property of fixing the cell in a life-like condition and the stains were in some way specific. The Benda and Kull methods of staining are not specific and are much more capricious than iron-alum haematoxylin in my hands and were abandoned early in the present study. Champy's fluid and Mottier's modification of Benda's Flemming fluid, the latter of which contains acetic acid, do not produce consistent adequate preservation of the mitochondria but with them one is forced to confine his observations to either peripheral or fundamental tissues depending upon the time of fixation, rate of penetration of the constituents of the fixing fluid, etc. It is admitted that the adequate fixation of plant tissues is a difficult thing, but one is hardly justified in interpreting that tissue which subjectively appeals to the investigator as being the correct image and to describe minutely morphological phenomena therefrom and insist upon the validity of such an interpretation for the other tissues of the same plant. This has been a common failing among the investigators of mitochondria. The different rates of penetration and fixation of the various constituents of the usual killing fluids produce a composite picture ranging from underfixation to overfixation, and since the fixing properties and the images produced by the constituents of a killing fluid vary greatly, the investigator is faced first of all with the decision as to which image he is going to accept. If one thinks of mitochondria in terms of narrow homologies, as Bowen and Guilliermond, and that there must be two categories of elements in the mitochondria of plants, it will be exceedingly difficult not to use the technic which best demonstrates what one is looking for and insist upon one's own interpretations. The literature is replete with mutual accusations of technical ineptness, whereas all seem guilty. Both Guilliermond and Bowen deprecate each other's work on grounds of technic. If the present paper be also guilty of a hypercritical attitude, it is not because the author believes he has reached the end of cytological technic but because he would like to shift the emphasis for mitochondrial investigations from repetitive morphological studies using demonstrably inadequate technics to the realm of technic itself since it seems obvious that no interpretation can be valid unless the observations are true. It should also be added that the evidence for the true nature of mitochondria in plants should be based not upon a certain tissue of a single species, as has often been the case, but upon observations which are true for all, or at least most plants, before theories concerning homology have any validity.

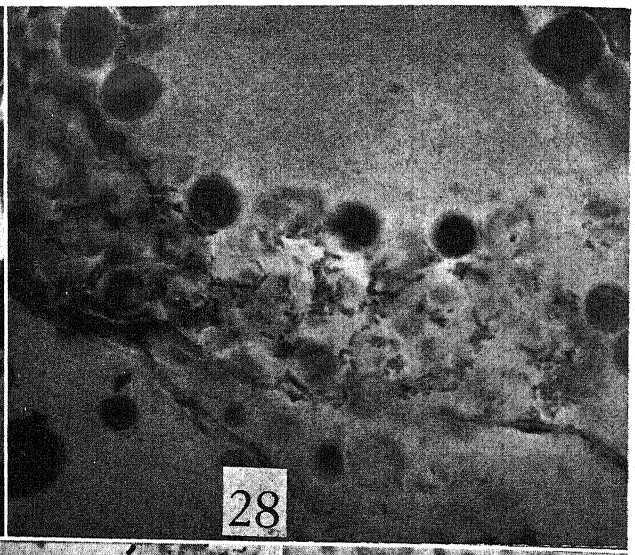
MITOCHONDRIA AFTER THE ALTMAN FREEZING-DRYING TECHNIC.—Staining of the mitochondria after the tissues were killed in liquid air and dehydrated *in vacuo* at —20°C. in the presence of P_2O_5 was very difficult unless mordanted for several days with potassium dichromate (fig. 39, 40, 41), but the technic, though presenting the investigator with very favorable material for microchemical studies, was not adequately exploited by the writer. The mitochondria and proplastids both gave a faint positive test for protein with Bensley's modification of the Millon reagent but were negative for fat with Alkanna, Scharlach red Sudan III, and Nile blue sulfate. They did, however, reduce osmium tetroxide in the same manner as in cells more conventionally prepared. Both mitochondria and proplastids before starch secretion were isotropic when observed with polarized light and crossed nicols.

FURTHER OBSERVATIONS.—Possible corroborative evidence is presented here for another function of mitochondria, related to enzyme activity. In the endosperm and scutellum of germinating seeds and similar tissues (fig. 27–29) the mitochondria were always observed to be numerous and in very close proximity to the amyloplasts being hydrolyzed. This view has been considerably developed by other workers: Newcomer (1940), O'Brien (1942). That these mitochondria are not reversion products is obvious because the amyloplasts are nearly all intact or showing only the usual lacunae concomitant with hydrolysis.

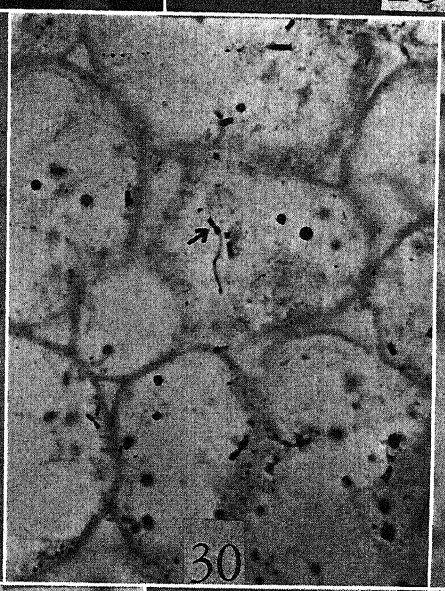
Two other observations are recorded. The first has to do with the structure of the mitochondria. Fig. 29 shows the mitochondria as being of a spiral nature with at least two relational coils reminiscent of chromosome structure. This observation was only made a few times and in all instances on corn. The other observation is that, in the reversion of plasts to mitochondria, the decrease in size is apparently sometimes effected by successive divisions of the plastids into mitochondria (fig. 31).

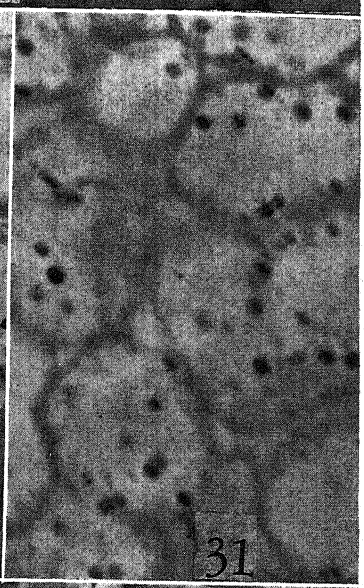
Fig. 21–26.—Fig. 21. *Narcissus* root-tip fixed as figure 3 showing plastid formation from polymorphic mitochondria. ×900.—Fig. 22, 23, 24, 26. Root-tips of *Pisum* after the Kolatchev technic showing the capriciousness of the technic. In figure 26 are seen the structures which correspond to the osmiophilic platelets of Bowen, whereas figures 22, 23, 24 show transition stages between these and a deformed mitochondriome. All tissues had the same treatment in the same vial. Bleached only and no stain. ×900.—Fig. 25. Root-tip of corn fixed by the modified Kolatchev technic but with a prolonged post-osmication (10 days) showing the reduction of OsO_4 in a typical predominantly granular pattern with increased time of treatment. ×900.

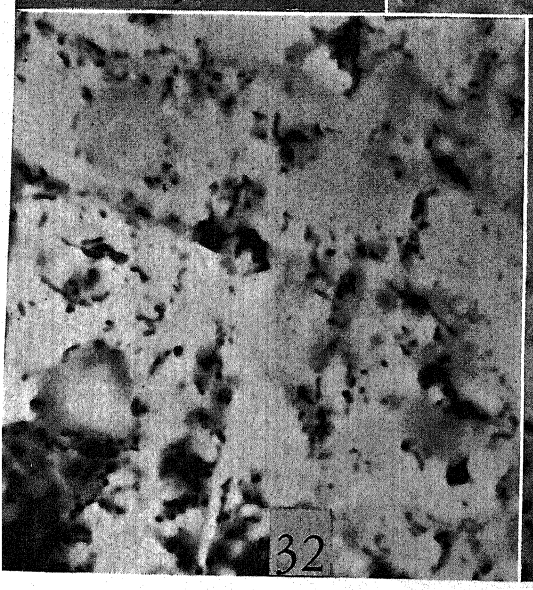

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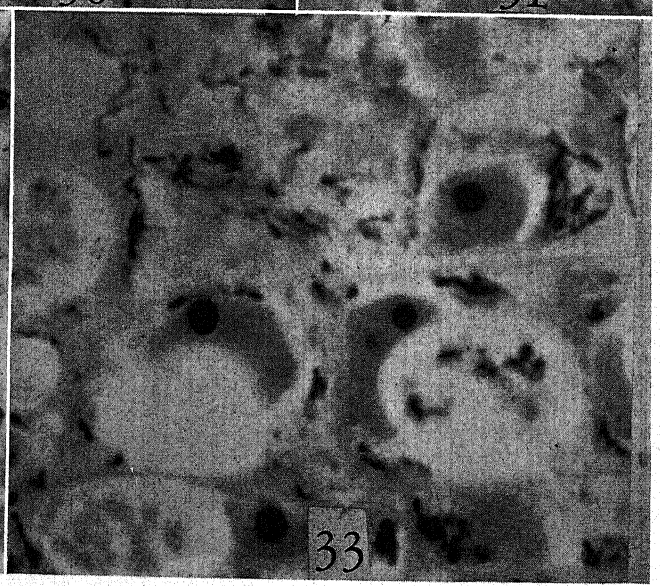

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THE OSMIOPHILIC PLATELETS. — According to Bowen (1927, 1928) the Kolatchev, Weigl and Mann-Kopsch impregnation methods, while they "give the most positive results on the osmiophilic platelets, they will also occasionally yield excellent results on both the plastidome and pseudochondriome." In my hands, the Kolatchev technic gave the most consistent results in the production of what Bowen described as the osmiophilic platelets. Fig. 26 corresponds most closely with his figures and descriptions of these bodies, but figures 22–24 also represent results of the same technic. These structures seem to be the products of a deformed mitochondriome, the degree of deformity varying with the conditions of fixation and the time and conditions of osmication. That the image produced by the Kolatchev technic is but a variably blackened image of the result of Champy's fixation in the Kolatchev technic seems clear. If Zirkle's fluid be substituted for Champy's (Newcomer, 1940a), the results are strikingly different and the mitochondria often appear selectively blackened, bleaching alone being necessary for their clear demonstration (fig. 20, 25, 37). Bowen (1929c) admitted that "the intensity of blackening is also subject to great variation, depending chiefly upon the length of osmication, and the intensity and frequency of artefacts both increase rapidly after the optimum osmication time has passed." How one can determine the optimum conditions for the preservation of a cytoplasmic constituent which has never been seen in a living cell and which is at best demonstrated in a variable manner by a capricious technic is an obvious question. The technics involving impregnation or the reduction of metallic salts such as silver and osmium have been found in this study to be so unpredictable and the results so variable in tissues fixed in the same bottle, that it would seem well to abandon them entirely for mitochondrial investigations. Practically any desired image can be secured and the results are confusion. Baker (1944) came to a similar conclusion and observed that the process of reduction throws the metal down in an elaborate network whose details are irrelevant, and that they constitute no guide to the selection of one image as true rather than another.

Since the validity of the osmiophilic platelets as a heretofore undiscovered category of cytoplasmic constituents rests upon results obtained by an admittedly and demonstrably capricious technic, it would seem well to await further investigations before accepting them as cell organs *sui generis*. From my own experiments and observations, it seems clear that they are the products of a deformed mitochondriome.

SUMMARY

The results of this study show that there are no distinctions in morphology, structure, chemistry, function, staining behavior, or behavior during mitosis among the mitochondria in plants whereby one can separate them into categories of organelles among which there is no genetic relation.

The two specific theories of the duality of the mitochondria in plants promulgated by Guilliermond and Bowen, respectively, are shown to be invalid. The results of this study and the literature on the subject suggest that the functions of mitochondria are multiple, variable or perhaps eclectic in nature, subject to the genetic and metabolic requirements of the organism.

The osmiophilic platelets are not cell organs *sui generis*, but artefacts of a deformed mitochondriome produced by capricious technics.

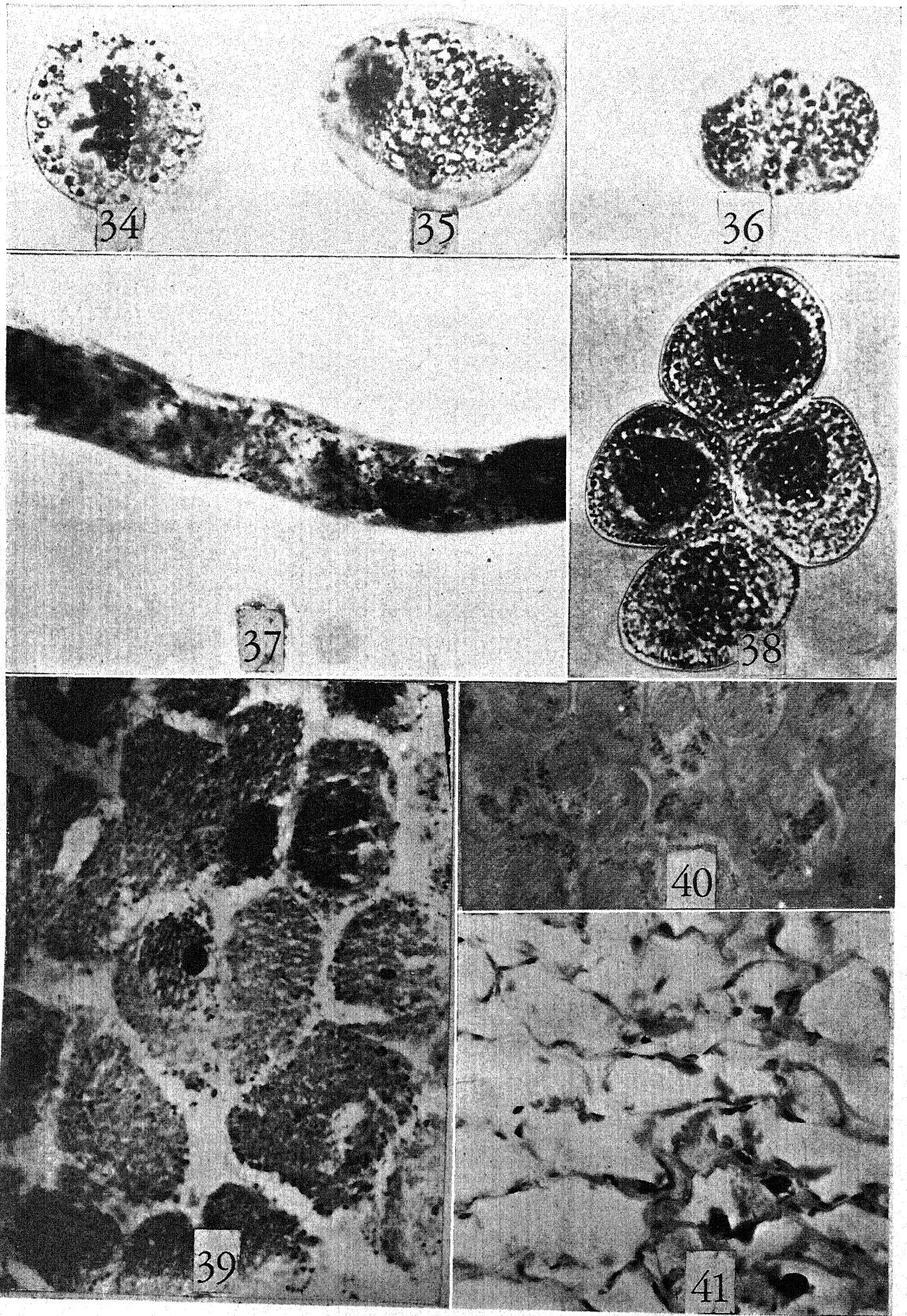
The heretofore mutually exclusive acid and basic fixation images have been combined to achieve complete fixation.

Suggestions for the correction and simplification of the terminology used in reference to mitochondria in plants are made as follows: (1) the term mitochondria (*mitos*, thread—*chondrion*, small grain) coined by Benda in 1897, should be retained on grounds of etymology, priority and common usage; (2) the term mitosome should be used logically in reference to the elongated or thread-like mitochondria, and the term chondriosome should be used in reference to the granular mitochondria; (3) the term mitochondriome should be used in a collective sense in reference to all the mitochondria of a cell or tissue.

Since none of the mitochondrial technics found in the literature gave adequate or consistent results, it is suggested that the emphasis for future mitochondrial investigations be shifted from morphological descriptions based upon inadequate technics to the subject of technic itself.

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Fig. 27–33.—Fig. 27. Somatic tissue adjacent to the archegonia of *Ginkgo* showing typical relation between mitochondria and amyloplasts during hydrolysis of the latter. Cf. figure 28. Zirkle's fixation. ×1350.—Fig. 28. Endosperm tissue of a three-day-old germinating wheat seed showing mitochondrial association with amyloplasts during starch hydrolysis. Zirkle's fixation. ×1350.—Fig. 29. Mitochondria in the scutellar tissue of a three-day-old germinating corn seed showing a peculiar spiral structure (not vesiculated) of the mitochondria. Observed only three times during this study and always in corn. Zirkle's fixation. ×900.—Fig. 30. Cells from the scutellum of a two-day germinated corn grain showing a peculiar bleb-like structure (see arrow) containing starch subtended by a mitochondrion apparently about to divide. Cf. figure 13. Fixation as in figure 1. ×900.—Fig. 31. Scutellar tissue of a two-day-old germinating seed of corn showing an apparent reversion of plastids into mitochondria by division. Zirkle's fixation. ×900.—Fig. 32. Root-tip of *Pisum* showing typical polymorphic mitochondria well preserved but the lack of nuclear detail characteristic of Zirkle's fixation. ×1350.—Fig. 33. Root-tip of *Narcissus* after Zirkle's fixation showing the relatively huge mitochondria occasionally observed. ×1350.


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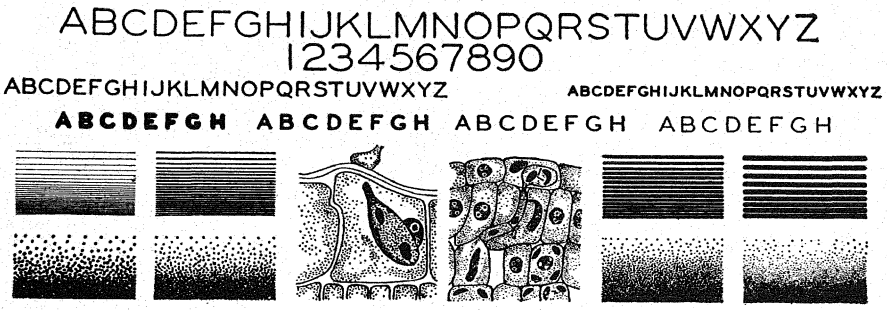
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Fig. 34–41.—Fig. 34. Aceto-carmine smear of *Ginkgo* pollen mother-cell at I metaphase showing peripheral distribution of chloroplasts. ×1350.—Fig. 35, 36. Telophase I stages of *Ginkgo* pollen-mother-cells showing plastid migration and orientation from a peripheral position into the spindle area where they align themselves in a row on each side of the new cell wall. ×1350.—Fig. 37. Pollen tube of *Begonia* grown and fixed *in situ* on slide (Newcomer, 1938). Fixation by modified Kolatchev. Bleached but unstained. ×1350.—Fig. 38. A quartet of *Ginkgo* microspores showing reversion of chloroplasts as seen in figures 34, 35, 36 to mitochondrial-like bodies. An aceto-carmine smear. Photographed about 1 minute after preparation. ×1350.—Fig. 39. Pollen mother-cells from *Lilium longiflorum* showing mitochondria. Fixation by the Altmann freezing-drying technic. Haematoxylin stain. ×1350.—Fig. 40. Pollen mother-cells of *Nicotiana rustica* fixed by the Altmann freezing-drying technic showing granular mitochondria (chondriosomes) surrounding the nuclei. Stained with iron-alum haematoxylin. ×1350.—Fig. 41. Somatic cells from another of *Nicotiana* fixed by Altmann freezing-drying technic showing transition of mitochondria into plastids. Stained with iron-alum haematoxylin. ×1350.





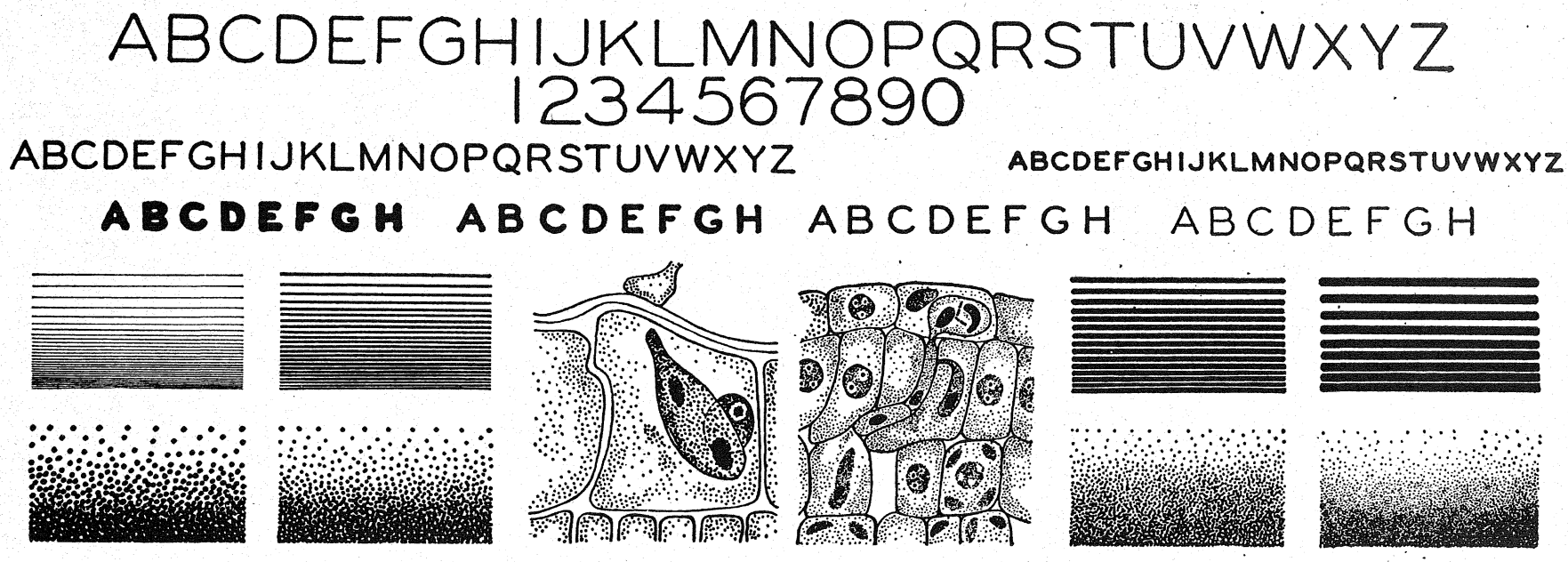
DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to ¼. Middle—Reduction to ½. Bottom—Original size.

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Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.



THE EFFECT OF MOISTURE STRESS ON NURSERY-GROWN GUAYULE WITH REFERENCE TO CHANGES IN RESERVE CARBOHYDRATES [1]

Hamilton P. Traub[2], M. C. Slattery[3], and W. L. McRary[4]

In a previous paper, Kelley, Hunter, and Hobbs (1945) reported on the effects of soil moisture stresses on nursery-grown guayule, *Parthenium argentatum* A. Gray, with respect to the amount and type of growth and subsequent growth responses on transplanting. The results included in the present paper are based on samples from a portion of the same experimental plots but are concerned with the effect of soil moisture stresses on changes in reserve carbohydrates, and 89 per cent ethanol solution levulins and 89 per cent ethanol insoluble levulins, in guayule tissues. This additional study was made possible by recent work which elucidated the nature of the reserve carbohydrates in the guayule plant (McRary and Traub, 1944; Hassid *et al.*, 1944; Traub and Slattery, 1946a, 1946b; Traub, Slattery, and Walter, 1946).

According to Traub (1946b), and contrary to the results reported by Spence and McCallum (1935), the rubber hydrocarbon in guayule apparently does not function as a reserve food. Although McRary and Traub (1944) and Hassid *et al.* (1944) reported the presence of inulin in guayule, Traub and Slattery (1946a, 1946b) showed that the main reserve carbohydrates in this plant are levulins, with inulin second in importance from the standpoint of total amounts present. Traub and Slattery (1946a, 1946b) have also shown that the measurement of the relative proportion of the 89 per cent ethanol soluble levulins, and 89 per cent ethanol insoluble levulins is a more sensitive tool in plant physiological research than the estimation of the total levulins and inulin. Since the free sugars are present in guayule stems and roots in relatively small amounts, Traub and Slattery (1946a, 1946b) conclude that the monosaccharides are not reserve materials but function as the carbohydrates of translocation. McRary and Slattery (1945) have reported on a method for the estimation of pentosans in guayule.

The detailed plan of the experimental plots and the methods for maintaining and measuring soil moisture stresses are included in the paper by Kelley, Hunter, and Hobbs (1945) and need not be repeated here in full. The following brief excerpts, pertinent to the present report, are quoted from that source:

"Guayule nursery seedlings were grown under five soil moisture stress ranges, . . . on the Chualar loam soil of the Alisal Nursery at Salinas, California. The moisture treatments were designed to maintain soil moisture stresses in the following ranges:

Treatment I, between the field capacity and a tension of 850 cm. of water at the 6-inch depth.

Treatment II, between the field capacity and a tension of 850 cm. of water at the 12-inch depth.

Treatment III, between the field capacity and the wilting point at the 6-inch depth.

Treatment IV, between the field capacity and the wilting point at the 12-inch depth.

Treatment V, regardless of the moisture stresses developed, the plants received no water after they were established.

The plants were seeded on June 3, 1943. Winter rains and pre-irrigation had wet the soil to a depth of at least 6 feet. Similar moisture treatments were given to all plots until July 13, at which time the plants were considered to be well established and differential moisture treatments were begun. . . . The differential moisture treatments were discontinued September 14. After that date moisture applications were uniform and due only to rainfall. . . . Each moisture treatment was randomly replicated eight times on plots 24 feet long and composed of two contiguous nursery beds, each having seven rows spaced 7 inches apart."

During the season 1943–44, Kelley, Hunter, and Hobbs (1945) made nine transplantings from all moisture treatments, Sept. 29 (I), Oct. 22 (II), Dec. 1 (III), Jan. 25 (IV), Mar. 8 (V), Apr. 5 (VI), May 3 (VII), June 2 (VIII), and July 8 (IX). At each transplanting, except the first and last, samples of the plants were prepared for carbohydrate analyses. Plant samples consisted of 12-inch sections across the five inside rows in the seven-row nursery bed, or 60 linear inches of row, and were harvested from that portion of the experimental area receiving 500 pounds of 10–15–5 fertilizer drilled in to a depth of 4 to 6 inches prior to seeding, and an additional 50 pounds of nitrogen per acre applied August 12. The individual plant units in the sample consisted of the tops plus a portion of the main root, approximately 6 to 7 inches long.

Methods of analysis.—The tissue samples were dried at 65°C., and finely ground as outlined by Traub (1946a). Two types of analyses were made, (a) analyses for free sugars, levulins, inulin, total

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water soluble carbohydrates, and pentosans; and (b) an estimation of the relative proportion of the 89 per cent ethanol soluble levulins, and the 89 per cent ethanol insoluble levulins. The analyses for free sugars, levulins, inulin, total water soluble carbohydrates and the relative proportion of the 89 per cent ethanol soluble, and insoluble levulins were carried out according to the procedure of Traub and Slattery (1946a), and the pentosan determinations were made according to the method of McRary and Slattery (1945).

The complete data are presented graphically in fig. 1 (water soluble reserve carbohydrates), fig. 2 (free sugars) and fig. 3 (pentosans). The changes

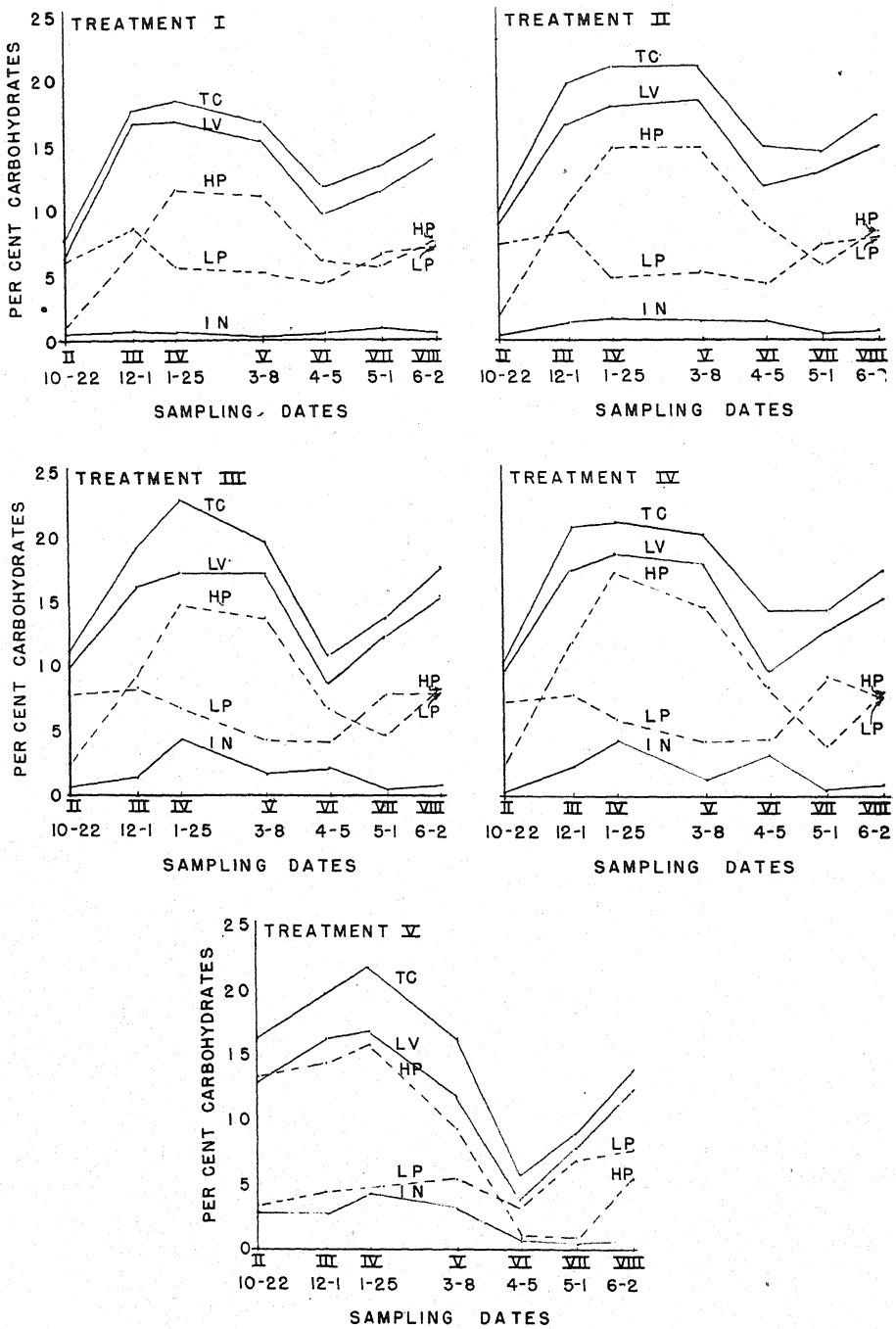


Fig. 1. Water soluble carbohydrate reserves of nursery-grown guayule plants grown under various moisture stresses; I, very low moisture stress, II, III, IV, intermediate moisture stresses, and V, high moisture stress. Carbohydrate reserves: total water soluble carbohydrates (TC); levulins (LV), inulin (IN), 89 per cent ethanol soluble levulins (LP), and 89 per cent ethanol insoluble levulins (HP). (See text for details.)

in these fractions, as affected by imposed moisture stresses and seasonal environmental conditions for the period of the study, are briefly considered in the following sections.

PRESENTATION OF DATA.—*Total water soluble reserves.*—Traub and Slattery (1946a) have shown that following the low in the spring with resumption of growth in guayule under normal conditions the total water soluble reserves are built up until mid-winter and then again begin to decline. This same trend is observed with guayule plants grown under various moisture stresses, but the total reserves (TC) built up for plants grown under low moisture stresses (treatment I) remain substantially below 20 per cent of the dry weight (fig. 1, A), whereas such reserves (TC) for plants grown under intermediate (treatments II, III, and IV) and high (treatment V) moisture stresses constitute well over 20 per cent of the dry weight at the winter peak (fig. 1).

Polysaccharides: levulins and inulin.—Traub and Slattery (1946a, 1946b) have shown that the levulins in guayule stems and roots always account for the major portion of the water soluble reserve polysaccharides, while inulin is relatively much lower in amount. These conclusions are borne out by the present data, as is shown in fig. 1. Irrespective of treatments, the levulins range from about 7 per cent to 18 per cent of the dry weight, while the inulin content varies from less than 1 per cent to about 4 per cent. Traub and Slattery (1946a) have also reported on the normal seasonal changes in reserve carbohydrates in guayule under conditions of the lower Salinas Valley, and show that both levulins and inulin rise to a peak during the winter, and fall to a low in spring. Thereafter the levulins rise, but the rise in inulin lags and is not marked until the temperature level is lowered in autumn. The data presented in fig. 1 for plants from plots subjected to intermediate soil moisture stresses (treatments II, III, and IV) generally bear out these conclusions. However, fig. 1, I, representing plants from plots subjected to lowest soil moisture stress, and fig. 1, V, representing plants from plots on which the highest soil moisture stresses were imposed, show different trends. With low moisture stresses (fig. 1, I) the inulin content is negligible even during the winter months and practically all of the water soluble reserves consist of levulins. In contrast, plants from the plots subjected to the most extreme soil moisture stresses, have almost reached their maximum levulins and inulin content by autumn.

Fractionation of levulins.—In contrast to the transformations indicated for total percentages of levulins and inulin, the changes in the relative proportion of the 89 per cent ethanol soluble levulins, and 89 per cent ethanol insoluble levulins are much more dynamic in seasonal physiological studies as previously reported by Traub and Slattery (1946a). Normally the 89 per cent ethanol soluble levulins (LP) decrease to a low in winter and increase to a peak in summer, whereas the 89 per cent ethanol insoluble levulins (HP) present a mirror image, rising to a high in winter and dropping to a low in summer. It should be noted from fig. 1 that for plants grown under low (treatment I) and intermediate (treatments II, III, and IV) moisture stresses the trends from one levulins fraction to another (LP to HP and vice versa) are similar, although absolute values may differ. However, for plants grown under high moisture stress (fig. 1, V), there is a radical departure in trend for 89 per cent ethanol insoluble levulins (HP) for they have nearly reached the level of the winter peak in October when these substances are still

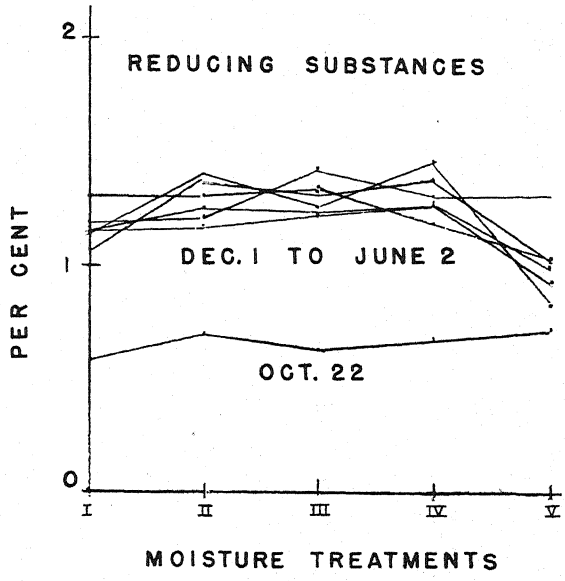


Fig. 2. Variation in total free sugars (reducing substances) in guayule plants from five different moisture treatment plots; each line represents one sampling date, Oct. 22 to June 2. (See text for details.)

relatively low for plants grown under low and intermediate moisture stresses (fig. 1, I, II, III, and IV).

Free reducing sugars.—According to Traub and Slattery (1946b) the free reducing sugar content of guayule stems and roots is relatively low, and this is borne out by the present data as shown in fig. 2. The free sugar content ranges from less than 1 per cent to 1.3 per cent of the dry matter. Although there appear to be tendencies for the free sugars to decrease with low (treatment I) and high (treatment V) moisture stresses as contrasted with the intermediate ones, no significant differences on the basis of Fisher's analysis of variance (Snedecor, 1944) could be demonstrated. These results apparently are in harmony with the conclusions of Traub and Slattery (1946b) that the free sugars are the carbohydrates of translocation since they are always present in relatively small amounts in the stems and roots of guayule plants. Constant utilization and conversion to polysaccharides ap-

TABLE 1. *The percentage of pentosans at six sampling dates in guayule seedlings grown under low and high moisture stress.*

Treatment	Sampling dates 10–22 (II)	12–1 (III)	1–25 (IV)	3–8 (V)	4–5 (VI)	5–3 (VII)	6–2 (VIII)	Mean	Difference required for significance P = 0.01[a]
I. Low moisture stress	1.74	1.41	1.69	...[b]	1.73	1.80	1.51	1.65	0.18
V. High moisture stress	2.31	1.94	2.06	...[b]	2.37	2.31	2.20	2.20	

[a] Since treatments differ by more than the required 0.18, the odds are greater than 99:1 (P = .0.01) that the differences were not due to chance.
[b] Samples lost during analysis.

parently keep the content of free sugars down to the minimum.

Pentosans.—The variation in pentosan content of guayule plants from the experimental plots is shown graphically in fig. 3. This indicates that

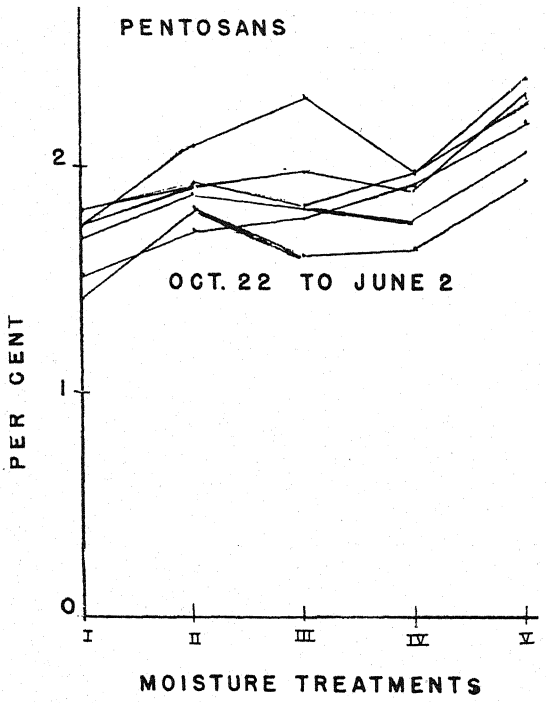


Fig. 3. Variation in pentosan content in guayule plants from five different moisture treatment plots; each line represents one sampling date, Oct. 22 to June 2. (See text for details.)

there is a general tendency for an increase in pentosans with increase in soil moisture stress. It is therefore not surprising that an analysis of variance shows a highly significant difference (P = 0.01) between treatment V (high moisture stress) and treatment I (low moisture stress), as shown in table 1.

The pentosan content of guayule plants was the only portion of the reserve carbohydrates determined that remained comparatively constant throughout the experiment even after the imposed soil moisture stresses had been removed. In contrast, the polysaccharides—levulins and inulin—are quite labile and undergo marked transformations due to changes in environmental factors.

DISCUSSION.—Now that the experimental data have been presented, it remains to consider their meaning in terms of growth responses and ultimate survival of transplanted nursery-grown guayule plants produced under five different moisture stress ranges. We need to note first, however, what limiting factors may be involved in rapid growth resumption on transplanting, and ultimate survival.

Top and root ratio.—There is a very great disproportion of tops to roots in guayule plants. According to Muller (1946), this ratio of top to root length in nursery plants is about 1 to 8 or more. The water absorbing area of the roots is therefore relatively much greater than the water transpiring area of the leaves. When plants are dug for transplanting only a relatively small portion of the root system is recovered and then the ratio is reversed making the water transpiring area (leaves) relatively much greater than the water absorbing area (roots). The recent work of Erickson (1945) has shown that the presence of leaves on nursery-grown guayule plants with reduced root lengths placed in water or planted in soil, leads to desiccation of the stem and root tissues. Such a moisture deficit in the plant apparently would operate as a limiting factor on growth resumption. It is true that in the usual field practice, part of the top (to 2 to 3 inches of the crown) is removed, but as a rule this does not remove all of the leaves. In the present experiment (Kelley, Hunter, and Hobbs, 1945), the plants were topped to within 1½ to 2 inches of the crown which removed all of the leaves, excepting for the first two transplantings when plants were topped to 2 to 3 inches of the crown. In these two transplantings, the topping did not remove all of the leaves, particularly from plants of treatment V. The change was made after the first two plantings when Smith (1944, 1945) showed that the presence of leaves on guayule plants after transplanting to the field retarded growth resumption.

Rainfall and temperature.—Guayule nursery plants are usually set in the field during the fall,

winter and early spring. In the lower Salinas Valley of California, the rainless season beginning in June is usually broken in October or November, but the greater amount of precipitation may not fall until mid-winter and early spring. Inadequate soil moisture may also be encountered in late spring or early summer transplanting unless irrigation water is applied. The transplanted nursery-grown plants may therefore be subjected to drought in the early fall and late spring and summer, and to low night temperatures during the fall and winter months. Such unfavorable seasonal conditions may constitute additional limiting factors to rapid growth resumption and survival of transplanted guayule seedlings.

Hardening process.—The work of Maximov (1926, 1928, 1929) and Waldron (1931) indicates that in general plants resistant to drought have the same general characteristics as those resistant to low temperature. According to Maximov (1926, 1928, 1929), resistance to low temperature and drought in plants is associated with accumulation in the tissues of sugars, other water soluble carbohydrates, and hydrophylic colloids. The "principal physiological peculiarity conditioning the endurance of plants to drought" according to Maximov, "is the capacity of enduring without permanent irreversible harm a considerable withdrawal of water from the plasma of the cells."

According to Tumanov (1927), the main role in the "hardening" process is performed by physiological changes within the cell, and anatomically such "hardening" leads to decrease in size of the newly formed cells and in general to the formation of a more xeromorphic structure. Addicott (1944), who studied some anatomical effects of moisture stress on plants from the same plots sampled in the present study, reports a decrease of the xylem and phloem areas with corresponding shorter rays and a decrease of the diameter of the pith and the size of its cells due to high soil moisture stress. However, he found no effect on "the thickness of the cork, the width of the vascular rays, the length of cells in the cortex or epidermis, the width of the cells in the cork or rays, or on the number of cells along the diameter of the pith." These results appear to be not entirely in harmony with those reported by Tumanov (1927), but the difference can apparently be explained on the basis that not all of the tissues of plants grown under treatment V were formed while the plants were under high moisture stress for all received similar moisture treatments (adequate moisture) until July 14. The high moisture stresses would be reached for treatment V only after a lag after the available moisture was depleted. Thereafter any anatomical effects from high moisture stresses would be apparent mainly in newly formed cells. The total observable anatomical effects would therefore not be as extensive as any internal physiological ones in the living cells of the plant in response to changes in the environment even after the tissues have been differentiated.

Carbohydrate reserves and growth responses.—Although Kelley, Hunter, and Hobbs (1945) report a progressive decrease in plant size and total dry weight with increases in moisture stress, within the ranges indicated (treatments II, III and IV), no marked progressive changes in water soluble carbohydrates are noticeable, either in total percentages of constituents, or in seasonal trends, except the lower inulin values for treatment II. For purposes of the present discussion therefore the data for plants from the intermediate moisture treatments, particularly treatments III and IV, may be considered together. From one irrigation to another, apparently, when the soil moisture declined from field capacity (16 per cent) to the wilting point (6 per cent) in these particular plots (II, III, and IV), the moisture available to the plants apparently enabled them to reach approximately the same development as far as percentage contents of water soluble carbohydrates are concerned. It is only within the extremely low (treatment I) and high (treatment V) ranges of moisture stress that there are marked departures from the indicated seasonal changes in water soluble carbohydrates (fig. 1), and in pentosan content (fig. 3) apparently due to differences in the available soil moisture supply.

According to Kelley, Hunter, and Hobbs (1945), various recorded effects due to soil moisture stresses imposed on guayule plants were still evident more than nine months later, although all plots in the experiment received identical moisture applications after September 14, 1943. According to these workers, guayule plants grown under high, in comparison with plants grown under low moisture stresses, gave better growth responses when transplanted. They resumed growth more quickly, grew more vigorously, in autumn transplantings gave a much higher percentage survival, and in all transplantings were comparatively better able to withstand unfavorable conditions after transplanting.

These authorities further state that, although plants grown under the lower moisture stresses (treatments I to IV, inclusive) with passing time showed improvement in growth responses, it was not until the fourth transplanting, in January, that plants of the first four treatments (I to IV, inclusive) survived transplanting at all satisfactorily, and not until the fifth transplanting in March that the growth responses were apparently comparable with those of treatment V, but even at this date growth was resumed much more slowly for plants from treatments I to IV, inclusive.

For purposes of discussion, the growth responses of the guayule plants, grown under five different moisture treatments, in relation to reserve carbohydrates in the tissues may therefore be conveniently taken up under the following time divisions, (a) second and third transplantings, Oct. 22 to Dec. 1; (b) fourth transplanting, Jan. 25; and (c) fifth to eighth transplantings, Mar. 8 to June 2.

a. Second and third transplantings.—During this period, as shown in fig. 1, for plant samples taken at the second and third transplantings (Oct. 22 and Dec. 1), those grown under high moisture stress (treatment V) contained relatively much greater percentages of 89 per cent ethanol insoluble polysaccharides (fig. 1, HP, and IN), and pentosans (fig. 3) as contrasted with plants from treatment I, and the intermediate treatments, II, III, and IV, all subjected to lower moisture stresses. These relatively higher percentages of water soluble carbohydrates, particularly 89 per cent ethanol insoluble levulins (fig. 1, HP) and pentosans (fig. 3), the latter apparently functioning as hydrophilic colloids, for plants grown under high moisture stress, are positively correlated with rapid growth resumption and a high survival percentage. In contrast, plants from low and intermediate moisture treatments, with lesser although rising percentages of 89 per cent ethanol insoluble levulins due to lowering of temperature in the fall (fig. 1, HP), and relatively lower pentosan contents, resumed growth more slowly and showed lower survival percentages as pointed out by Kelley, Hunter, and Hobbs (1945).

Earlier in this paper reference was made to the fact that due to topping to 2 to 3 inches of the crown, some of the leaves were not removed from plants of the second transplanting, particularly in the case of treatment V. In spite of the presence of leaves, plants from treatment V, grown under high moisture stress, were first in resuming growth. Apparently the greater percentages of 89 per cent ethanol insoluble levulins and pentosans, the latter functioning apparently as hydrophilic colloids, were effective in preventing any undue desiccating effect due to transpiration from the leaves that remained on the plants.

b. Fourth transplanting.—At the fourth transplanting, in January, the 89 per cent ethanol insoluble levulins (fig. 1, HP) reach a comparable maximum in treatments II, III, IV, and V. During this same period the inulin values (fig. 1, IN) rise highest for treatment V, with treatments III and IV next highest, followed in turn by treatments II, with only a slight rise, and treatment I, with no rise in inulin. Plants subjected to the lowest moisture stresses (treatment I) apparently had lost their capacity to accumulate inulin in response to low air temperature due to the previous soil moisture treatment.

It should be noted that Kelley, Hunter, and Hobbs (1945) point out that it was not until the fourth transplanting, in January, that plants of the first four treatments (I to IV, inclusive) survived transplanting at all satisfactorily. Apparently, maximum growth resumption of the guayule plants on transplanting is due to multiple factors, including the presence of optimum percentages of 89 per cent insoluble levulins and pentosans as the limiting ones under the seasonal conditions of temperature and moisture. In January, plants grown under high moisture stress (treatment V), from the standpoint of carbohydrate reserves differed from plants grown under intermediate moisture stresses (II, III, and IV) mainly on account of a significantly higher pentosan content (fig. 3). The continued superiority in growth responses of plants from treatment V is apparently due to the greater hydrophilic colloid content (pentosans). Plants from treatment I, lowest in 89 per cent ethanol insoluble levulins and also low in pentosans, were most backward in growth resumption as shown graphically by Kelley, Hunter, and Hobbs (1945; fig. 6, page 208).

c. Fifth to eighth transplantings.—At the fifth transplanting in March, the water soluble carbohydrate values for all treatments, I to V, inclusive, are either at the end of a peak or have begun to decline to the spring low. At this stage, and thereafter to the end of the experiment in early June, plants from the high moisture stress plots (treatment V) differ from those from the low and intermediate stress plots (treatments I to IV, inclusive), as far as reserve carbohydrates are concerned, in having a significantly higher pentosan content (fig. 3). Kelley, Hunter, and Hobbs (1945) point out that it was not until the fifth transplanting in March that growth responses of treatments I to IV, inclusive, were comparable with those of treatment V, but even at this date growth was resumed much more slowly for plants from treatments I to IV, inclusive. It has already been indicated that such superiority of growth responses of plants from treatment V apparently is due to their significantly higher pentosan content. It is during this period, Kelley, Hunter, and Hobbs (1945) point out, that plants of treatment V in the nursery bed "sent forth new shoots several weeks earlier than plants of any other treatment and . . . . their growth was much more vigorous." The effect of this on reserve carbohydrates is indicated in fig. 1 which shows that these reserves began to decline earlier and at a more rapid rate. Kelley, Hunter, and Hobbs (1945) further show that "Relatively, the treatment V plants produced nearly 3 times as much growth during the spring of 1944 as the plants of any other treatment." The effects of this are indicated in fig. 1 which shows that the carbohydrate reserves for treatment V plants decline relatively much more than for plants of any of the other treatments.

SUMMARY

Analyses were made of total water soluble carbohydrates, free sugars, levulins, inulin, the relative proportion of 89 per cent ethanol soluble levulins and 89 per cent ethanol insoluble levulins, and pentosans in guayule plants grown under soil moisture stresses ranging from very low to very high in order to discover any correlations between (a) changes in carbohydrate reserves in the plant tissues and moisture tension during the growing period, and (b) the carbohydrate reserves of plants

and their subsequent growth responses and survival after transplanting in the field.

Guayule plants grown under high moisture stress in comparison with plants produced under low and intermediate moisture stresses, (a) in October, contained relatively greater percentages of total water soluble carbohydrates, including 89 per cent ethanol insoluble levulins and inulin, and pentosans; (b) retained a significantly higher pentosan content for the duration of the experiment (to June); (c) began to utilize the water soluble carbohydrate reserve earlier in the spring and to a relatively greater degree.

For plants grown under high moisture stress, (a) the relatively higher percentage of water soluble carbohydrates in October was correlated in fall transplantings with more rapid growth resumption and greater percentage survival; (b) the retention of relatively higher pentosan percentages in the tissues was correlated with more rapid growth resumption throughout the experiment; and (c) the earlier and more extensive utilization of the water soluble carbohydrate reserves with earlier growth resumption, and relatively more vigorous growth, resulted in a relative production of nearly three times as much growth during spring as was produced by the plants of any other treatment.

For plants grown under the intermediate and high moisture stresses, the effect of the relatively lower temperatures in fall and winter was similar to that of very high moisture stress during the growing season, leading in both instances to increases in the percentage of total water soluble carbohydrates, 89 per cent ethanol insoluble levulins and inulin of the plant tissues.

Plants grown under very low moisture stress apparently lost the capacity to accumulate inulin during the following fall and winter in response to lower temperatures, but retained the capacity for the accumulation of relatively lower percentages of total water soluble carbohydrates and 89 per cent ethanol insoluble levulins in response to lower temperatures.

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PHYSIOLOGICAL STUDIES OF GELIDIUM CARTILAGINEUM. I. PHOTOSYNTHESIS, WITH SPECIAL REFERENCE TO THE CARBON DIOXIDE FACTOR [1]

C. K. Tseng and Beatrice M. Sweeney

GELIDIUM CARTILAGINEUM is a red alga with repeatedly pinnately branched, maroon-purple fronds. In southern California it is represented by the variety *robustum,* which grows most abundantly on submarine rocks 10 to 40 or more feet below sea level, and which may reach a length of 4 feet. This plant is the principal agarophyte for the manufacture of agar on the Pacific coast of North America.

Agar shortage in the last few years has stimulated considerable interest in the study of agarophytes. The importance of raw materials in an industry cannot be over emphasized. The success of the American agar industry will depend, in large measure, on the adequacy of the supply of agarophytes. A knowledge of their physiology and ecology is prerequisite to finding ways of insuring an adequate natural supply of these seaweeds, or, if necessary, of cultivating them on a commercial scale. A program of research on agarophytes has therefore been inaugurated at the Scripps Institution of Oceanography. The present report, the first of a series, is concerned with the photosynthetic activities of this *Gelidium.*

Photosynthesis is the fundamental process providing both energy and substance for the synthesis of the whole plant organism. It follows that in studies of photosynthesis are to be found solutions to many problems concerning the ecological behavior of plants. It is therefore the object of the authors to study the photosynthetic activity of *Gelidium cartilagineum* under different environmental conditions, and especially to determine the effect of varying light, temperature, and carbon dioxide concentration in sea water, in order to obtain some idea of the optimum conditions for the growth of this particular plant.

THE CARBON DIOXIDE FACTOR.—Since photosynthesis is a process requiring carbon dioxide as a substrate, the rate of photosynthesis cannot be constant over a period of time unless carbon dioxide is supplied at a constant rate, or enough is continually present to saturate the enzyme system involved. It has been well established that carbon dioxide is a limiting factor in the photosynthesis of land plants during the day, since the photosynthetic activity of leaves is greatly enhanced by increasing the carbon dioxide concentration in the air. In the case of algae and aquatic plants under natural conditions, *e.g.,* filamentous algae and phytoplankton in particular, it has been established that a high carbon dioxide concentration leads to a dense plant population, indicating greater photosynthetic activity. In shallow water carbon dioxide is almost the sole limiting factor in photosynthesis during a bright sunny day (*cf.* Burr, 1941). In laboratory studies of photosynthesis of green algae such as *Chlorella,* carbon dioxide tension has been shown to be a limiting factor (Emerson and Green, 1938; Smith, 1938).

While it is to be expected that the red algae behave as do other chlorophyllous plants toward carbon dioxide tension, very little experimental work has been done on this subject. In fact, the only critical work to our knowledge is that by Emerson and Green (1934). These authors found that in *Gigartina harveyana* the rate of photosynthesis was a function of carbon dioxide concentration up to about 360×10^{-6} mole per liter, a concentration about 36 times higher than that found in natural sea water. They called attention to the importance of maintaining the carbon dioxide concentration above the limiting value when the effects of other factors on photosynthesis are to be determined. This has been a fault, as they showed, in most of the early work on marine algae subjected to so-called "natural" conditions, in which a large amount of the plant material was enclosed in a relatively small volume of sea water, and photosynthesis was measured over relatively long periods. In the course of the experiment, the available carbon dioxide became exhausted, so that at some undetermined time the rate of photosynthesis began to fall, resulting in low values.

It seems probable from the above considerations that the concentration of carbon dioxide in sea water in the La Jolla region may not be sufficient to maintain photosynthesis of *Gelidium cartilagineum* at a uniform rate in a closed system for any prolonged period of time. It was therefore the object of our experiments first to determine the amount of material to be used and the concentration of carbon dioxide which should be present in sea water in order to maintain photosynthesis under specific conditions at a constant rate; and secondly, to apply the results to the study of this alga in the laboratory as well as under natural conditions. In order to determine whether the carbon dioxide present in ordinary sea water is sufficient to maintain a constant rate of photosynthesis, comparative studies were conducted on photosynthesis in sea water with and without carbon dioxide from outside sources.

A simple way of increasing the carbon dioxide concentration is to add a bicarbonate, *e.g.,* sodium or potassium bicarbonate. This, in solution, is in

[1] Contributions from the Scripps Institution of Oceanography, University of California, La Jolla, California, New Series, No. 296.

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equilibrium with free carbon dioxide (CO_2) according to the following equation:

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^- \rightleftarrows H^+ + CO_3^=.$$

As CO_2 is removed, it will be replaced from the bicarbonate (HCO_3^-). The amount of CO_2 at any moment depends on the total carbonate and the pH and, as Emerson and Green (1934) described, may be calculated if the total salt content (salinity) is known.

Since in our experiments the carbonate ion concentration was unknown, we have rearranged the equations of Emerson and Green as follows:

$$[CO_3^=] = \frac{K'_2}{K_{C1}} \cdot \frac{[HCO_3^-]^2}{[CO_2]}$$

$$[CO_3^=] = \frac{K'_2\,[HCO_3^-]}{[H^+]}$$

$$i.e.\ [CO_2] = \frac{[HCO_3^-]\,[H^+]}{K_{C1}}$$

in which K_{C1} is the carbon dioxide concentration constant and K'_2 the second dissociation constant of carbonic acid in sea water. For purposes of calculation, the HCO_3^- was obtained by subtracting from the total carbon dioxide, or $CO_2 + HCO_3^- + CO_3^=$, ($\Sigma\ CO_2$), as determined by the Van Slyke apparatus, the amount of $CO^=_3$ present in sea water at the pH of the solution as given by Moberg *et al.* (1934, p. 269, fig. 6). At pH 7.5 or above, the $[CO_2]$ is so low, in comparison with the $\Sigma\ CO_2$, that for practical purposes, it may be neglected in the calculation. The $[HCO_3^-]$ remains the same at pH 7.5 and below. Since the pH shifted continuously toward the alkaline side as carbon dioxide was assimilated in the course of photosynthesis, the average of the $[H^+]$ at the beginning and at the end of the experimental period was taken for the value of $[H^+]$. These were calculated from the respective pH values. The CO_2 contents of some of the solutions, thus calculated, are given in table 1.

MATERIAL AND METHODS. — *Material.* — Unlike *Gigartina harveyana,* a broad membranous form used by Emerson and Green (1934), *Gelidium* is fern-like with pinnate branches of several orders. It is impossible to select identical samples with the same area. Dry weight of the plant, instead of its area, is therefore used as the basis for comparison. In earlier experiments, samples with fresh weights of about 2.5 to 3.0 g. were used. These gave oven-dried weights of about 0.6 to 0.7 g. Since the volume of water used was about 550 ml. in those experiments, these were equivalent to 5 to 6 g. of fresh material in a liter of solution. This is only about 1/10 of the material used by Ehrke (1931) and about 1/3 of that used by Emerson and Green (1934).[2] Still, this amount of material proved to be too much. Therefore in later experiments, samples with dry weights of 0.1 to 0.2 g. were used and 270 ml. bottles employed. Samples were chosen in such a way that they appeared as nearly alike as possible. Either the entire frond of a young plant or the ultimate branches of an older one were used. They were thoroughly washed and brushed to free them from any adhering organisms or detritus. At the end of an experiment, the samples were oven-dried at 110°C. for about 6 hours, allowed to cool in a desiccator, and weighed.

Method.—Emerson and Green (1934) used Warburg's technique[3] in their experiments, while Ehrke (1931) and other workers (Kniep, 1914; Harder, 1915; Montfort, 1929; Tschudy, 1934) used the "bottle" method, in which narrow-mouthed bottles or tubes were filled to the neck with water, stoppered tightly, and exposed to light for a certain length of time. The oxygen content of the water was determined before and after illumination, and the production of oxygen used as an indicator of the photosynthetic activity. There is no doubt that the "bottle" method is much less critical than the manometric method. For ecological-physiological experiments conducted outdoors, however, this seems to be the only practical method. As pointed out above, workers employing this method have generally used too much material in a relatively small volume of water, the carbon dioxide tension of which was evidently too low to maintain a constant rate of photosynthesis. We have therefore modified the method to eliminate these and other defects so that it may be adapted to outdoor work under natural conditions as well as laboratory experiments under controlled conditions. This modified "bottle" technique may be outlined as follows:

A piece of *Gelidium* is introduced into a pyrex bottle provided with a ground glass stopper and containing two glass balls. The bottle is filled to the neck with water siphoned from a carboy. The stopper is slipped in place and tightened, and the bottle is placed on a shaker. For determining the respiration of *Gelidium*[4] in each water sample, a bottle containing a piece of the alga and enclosed in dark cloth is included. When all bottles are ready, light is turned on and shaking started. At the end of the experimental period, the bottles are inverted several times and three water samples are siphoned from each bottle for the determination of the oxygen, total carbon dioxide content and pH.

[2] Ehrke (1931) used as much as 50 g. of fresh material in a liter of sea water, and Emerson and Green (1934) about 150 mg. in 8 ml. of sea water, or approximately 19 g. per liter.

[3] For purposes of comparison, we have conducted some preliminary experiments using Warburg's indirect method. The only manometric apparatus available to us required lighting from above. In making simultaneous determinations of P_{CO2} and P_{O2} during photosynthesis of this alga, we found that there was a long lag in the pressure changes in the gas phase on changing from dark to light. This made determinations of photosynthesis of *Gelidium,* using our Warburg apparatus, impractical.

[4] In the earlier experiments, the same plants were used in the determination of photosynthesis and respiration. Pairs were employed, one of which was lighted and the other darkened in the first period. These were reversed in the sceond period.

The initial oxygen and total carbon dioxide contents as well as pH of the water were determined at the beginning of the experiments. Changes in oxygen and carbon dioxide in both light and dark bottles can then be calculated. The sum of the oxygen produced in the light and consumed in the dark, reduced to milligrams of oxygen per gram dry weight of plant per hour, gives the photosynthetic activity of the particular specimen under investigation.

Water.—Sea water which had been filtered with suction through a fritted glass filter of medium pore size was used in the earlier experiments. This water was not further sterilized, but small changes in the oxygen and carbon dioxide content of control water samples made it evident that some mannoplankton and bacteria passed through the filter. The effect of these organisms on the experiments was small, but in later experiments was eliminated by autoclaving the filtered sea water at 121°C. for 20 minutes. Autoclaving, however, reduces the amount of ΣCO_2 and increases the pH of the water (table 1). Therefore, later experiments, water was filtered twice, first through fine filter paper and then through fritted glass filter of fine porosity. Practically all the phytoplankton and bacteria were thus removed and the ΣCO_2 and pH of the water not much affected. To the sea water were added various amounts of $KHCO_3$ shortly before the start of the experiment. In some experiments, $NaHCO_3$ was used. No difference was found in the effects of sodium and potassium salts on photosynthesis during the few hours of experiments.

The ΣCO_2 content of all water samples was determined. The ΣCO_2 of filtered sea water was found to vary between 89.5 and 93.3 mg. per liter (average 91.3 mg./l.), a value in good agreement with the data of Moberg *et al.* (1934). The ΣCO_2 content of autoclaved filtered sea water was lower, ranging from 72.7 to 83.1 mg. per liter (average 76.7 mg./l.). In natural sea water it was impossible, by addition of bicarbonate, to increase the free carbon dioxide above *ca.* 40×10^{-6} mole per liter without precipitating calcium and magnesium carbonates. By lowering the pH, however, this may be accomplished, since $[CO_2]$ may be increased by increasing the $[H^+]$ as well as the $[HCO_3^-]$ (*cf.* equations given above). Osterhout and Dorcas (1925), in their experiments on the penetration of CO_2 into *Valonia* cells, have employed dilute HCl to lower the pH of sea water in order to increase the $[CO_2]$. In our experiments the addition of about 9 to 10 ml. of 0.1 N HCl to a liter of filtered sea water lowered its pH from 8 to about 6.5, while twice as much acid was necessary to bring about similar lowering of pH in the presence of 0.004 moles of bicarbonate per liter because of the buffering capacity of this salt. The introduction of additional chloride, which is already present in large quantity in sea water, should not produce any toxic effects. The amount of additional chloride ion did not exceed 0.4 per cent of that originally present.

Potassium dihydrogen phosphate, up to 0.001 mole per liter was also used to lower the pH of the solutions. No toxic effect on the plant was observed in the first two hours. In fact, there appears to be higher rate of photosynthesis in the presence of additional phosphate. In the third hour, however, a definite decline in activity was noted (table 3). Moreover, the buffering capacity of the acid phosphate solutions in the closed system was only slightly better than sea water acidulated with HCl. Therefore, HCl was preferred as the pH-lowering agent. By the addition of HCl and $KHCO_3$, the $[CO_2]$ was increased more than thirty times at pH 7 (table 1).

Bottle and shaker.—As in the regular "bottle" method, glass-stoppered pyrex bottles of about 270 ml. capacity were used to hold the plant material. In some experiments, larger bottles (550 ml.) were used, but the results were the same. Since agitation has been found by one of us (Tseng) to be an important factor in the growth of this particular plant in culture experiments, provisions were made for agitating the solutions by placing two glass balls of about 1 cm. diameter in each bottle. The presence of the balls also insured a more even distribution of the dissolved gases when, at the end of the experiment, the bottles were inverted several times before water samples were siphoned off.

TABLE 1. *Free carbon dioxide in various seawater samples.*[a]

Water sample	pH	$[H^+]$, moles/liter $\times 10^{-8}$	$[HCO_3^-]$, moles/liter $\times 10^{-3}$	$[CO_2]$, moles/liter $\times 10^{-6}$
Autoclaved seawater	8.47	0.34	1.0	3.2
Filtered seawater	8.10	0.79	1.5	11.3
Autoclaved seawater + 0.004 M $KHCO_3$	8.25	0.57	3.7	20.1
Filtered seawater + 0.004 M $KHCO_3$	8.00	1.00	4.0	38.2
Filtered seawater + HCl	7.00	10.00	1.5	143.0
Filtered seawater + 0.004 M $KHCO_3$ and HCl	7.00	10.00	4.0	382.0

[a] Calculations based on the formula $[CO_2] = \frac{[HCO_3^-]\,[H^+]}{K_{c1}}$ (see text), in which $K_{c1} = 1.047 \times 10^{-6}$, since $pK_{c1} = 5.98$ at Cl = 19.00, T = 18°C. according to Buch *et al.* (1932).

The bottles were placed horizontally on a shaker, the platform of which could accommodate twenty 270-ml. bottles or eight 550-ml. bottles. The shaker platform moved through a distance of 12.5 cm. and made 72 complete excursions per minute. In earlier experiments, the platform was arranged to tilt, instead of moving in a horizontal plane, but this was less desirable because of changes in the light intensity thus introduced.

Light.—As light source, "daylight" fluorescent lamps were used. In recent years, such lamps have been used quite extensively by botanists as a source of light for experimental work and for the culture of higher plants. Although their use has not been reported in work on marine algae, preliminary experiments showed their suitability for the present study. A bank of eight 36-inch, 30-watt daylight lamps was placed about 8 inches above the bottles. The reflecting surface of the bank was painted white. The light intensity of all the bottles was not exactly the same, but the differences did not exceed 20 foot-candles. When the lamps were first used, the light intensity on the bottles was about 800 foot-candles or 8600 lux; after several experiments, it dropped to about 750 foot-candles or 8000 lux. This intensity was chosen since it is approximately the illumination of average coastal waters at a depth of 10 meters (Sverdrup *et al.*, 1942; p. 776), where *Gelidium* grows most luxuriantly. Light intensities were measured with a standardized Weston photometer, model 614.

The entire process of preparing the bottles, siphoning water into the bottles, wrapping some in dark cloth, and placing them on the shaker, required 15 to 20 minutes. Thus, before the experiment was begun, some bottles unavoidably stood filled and closed longer than did others. During this time gas exchanges, of course, took place. However, error from this source was insignificant. In a preliminary experiment it was found that, when the two room lamps (about 3 feet from the work table) were lighted, the light intensity on the work table was slightly below the compensation point for this *Gelidium,* since there was a net decrease in oxygen content per bottle of 0.03 mg. per liter per hour, or less than 0.01 mg. per liter bottle for the entire preparation period. This amount of oxygen consumed is within the experimental error of the method of oxygen determination and may therefore be neglected.

Temperature.—Experiments were carried out in a constant temperature room with a temperature of $16\pm0.5°C$. Although little heat was radiated from the fluorescent lamps, it raised the temperature inside the experimental bottles about 2°C. above that of the room by the end of the 3-hour experimental period, *i.e.,* to $18.5\pm0.5°C$. The latter temperature was chosen since field experience has shown that *Gelidium* grows best when the temperature of the sea water is about 18°C.

Determination of O_2, ΣCO_2 and pH in sea water sample.—In our experiments the dissolved oxygen in water samples was determined by the Winkler titration method, which is widely used among hydrobiologists. In order to avoid the correction of the gas volumes to standard conditions, we have determined oxygen by weight. A N/80 thiosulfate solution was used since one ml. of this reagent is equivalent to 1 mg. O_2 in the water sample.

ΣCO_2 dissolved in the sea water samples were determined by the Van Slyke manometric technique, using the method of Greenberg, Moberg and Allen (1932). Duplicate determinations were made and found to agree to within 0.5 mg. ΣCO_2 per liter. Concentrations up to 300 mg. per liter were successfully measured by this method.

All pH measurements were made with a glass electrode using a Beckman pH meter, model G. The pH of the solutions were measured at the beginning and at the end of each experimental period. The average pH for any experimental period was calculated from the average $[H^+]$ for that period.

Sources of error.—Errors involved in the determination of photosynthesis by this method may be traced to several sources. There are technical errors in the chemical determination of the oxygen and carbon dioxide amounting to about 1–2 per cent. When dry weights of the samples did not exceed 600 mg. per liter of sea water, small differences in amount of plant material used did not introduce significant errors. There was, however, a considerable difference in the rate of photosynthesis of different plant samples under identical experimental conditions. This source of error arises from inherent differences in the plant material, impossible to avoid when standard cultures are not available. Some part of this variation may be due to differences in the relative amount of subcylindrical stem-like portion of the frond, since this, presumably, is not so active photosynthetically as the flattened leaf-like portion. An attempt to avoid this source of error basing calculations on chlorophyll content rather than on dry weight will be reported in a later paper.

Discussion of results.—There are several problems concerning carbon dioxide as a limiting factor in photosynthesis of *Gelidium cartilagineum.* First of all, there is the question whether natural sea water contains enough carbon dioxide to prevent limitation of this physiological process by this factor. So far, the only critical work on this subject has been that of Emerson and Green (1934). Experiments were therefore conducted in which the rates of photosynthesis in both natural and bicarbonate-supplemented sea water were compared. A series of $KHCO_3$ concentrations up to 0.005 M was used. Higher concentrations were not employed because of the precipitation of calcium and magnesium carbonates. Emerson and Green (1934) avoided this difficulty by using artificial sea water which was made to contain less calcium and magnesium than does natural sea water. They were

TABLE 2. *Photosynthesis at different* ΣCO_2. *Total carbon dioxide concentration in sea water increased by addition of* $NaHCO_3$. *Data from Experiment 44–12. Light intensity 880 f. c. Experimental period, 3 hrs. (T).*

ΣCO_2 mg./l.	Plant	W weight of plant in grams	V volume of bottle in liters	$\Delta O_2'$ O_2 produced in mg./l.	$\Delta O_2''$ O_2 consumed in mg./l.	O $= \Delta O_2' - \Delta O_2''$	OV $R_1 = \frac{O}{WT}$ Rate of photosynthesis in mg. O_2/g./hr.	$\Delta CO_2'$ CO_2 consumed in mg./l.	CO_2 produced in mg./l.	C $= \Delta CO_2' - \Delta CO_2''$	CV $R_2 = \frac{C}{WT}$ Rate of photosynthesis in mg. CO_2/g./hr.	Average rate of photosynthesis in mg. O_2/g./hr.	Average rate of photosynthesis in mg. CO_2/g./hr.
82.5	A	0.6170	0.5445	9.78	—1.00	10.98	3.17	—13.30	1.62	—14.92	—4.39	310	—4.34
	B	0.6455	0.5449	10.35	—0.44	10.79	3.03	—14.17	0.72	—14.89	—4.29		
147.5	C	0.6408	0.5439	13.75	—1.04	14.79	4.19	—20.88	1.36	—22.24	—6.30	4.27	—6.31
	D	0.7018	0.5262	16.11	—1.24	17.35	4.35	—23.14	2.04	—25.18	—6.32		
190.2	E	0.6677	0.5496	20.35	—1.11	21.46	5.89	—30.98	2.16	—33.14	—9.09	5.49	—8.03
	F	0.5974	0.5513	15.46	—1.04	16.50	5.08	—21.42	1.22	—22.64	—6.97		
297.6	G	0.6932	0.5442	22.84	—1.06	23.90	6.25	—35.43	2.40	—37.83	—9.90	6.20	—9.19
	H	0.6658	0.5441	21.50	—1.02	22.52	6.10	—29.72	1.35	—31.07	—8.47		

thus able to add as much as 0.015 M HCO_3^-. Natural sea water was used in our experiments, however, since it was not known what role the traces of minor elements and other substances, absent in artificial sea water, may play in the photosynthesis of *Gelidium*.

The rate of photosynthesis was greatly accelerated by the addition of the bicarbonate. In table 2, the results of a single experiment are given in which relatively heavy samples of about 0.6 g. per half liter were used for determining the rate over a 3-hour period. There was a 100 per cent increase of photosynthesis from 3.1 mg. O_2/g. sample/hr. in sea water with 82.5 mg. ΣCO_2/l. to 6.2 mg. O_2/g. sample/hr. in bicarbonate-supplemented sea water with 297.6 mg. ΣCO_2/l. In sea water having intermediate amounts of ΣCO_2, the rates were similarly intermediate. Evidently, natural sea water does not provide a sufficient amount of carbon dioxide to saturate the enzyme system for photosynthesis of *Gelidium*.

Analyses of the results from several experiments showed dependence of the rate of photosynthesis on the weight of the sample used. When the rates were plotted against ΣCO_2, it was noted that the data fell on two curves (fig. 1). Samples weighing 0.3 to 0.6 g. per liter sea water (Curve B) have consistently higher rates of photosynthesis that those weighing over 0.6 g. (Curve A). The rates of the heavier samples increased from about 3 mg. O_2/g. sample/hr. in sea water to 6 mg. O_2/g./sample/hr. in sea water containing 0.005 M HCO_3^- (*ca.* 300 mg. ΣCO_2/l.), while those of the lighter samples increased from 6 to 8 mg. O_2/g./sample/hr. with a corresponding change in ΣCO_2. A flattening of Curve B is apparent, when the ΣCO_2 concentration reaches 250 mg./l. (water containing *ca.* 0.004 M HCO_3^-). Increase of the ΣCO_2 concentration above this point did not increase photosynthesis appreciably.

The dependence of photosynthesis on the amount of plant material as well as on the ΣCO_2 concentration probably indicates a depletion of carbon dioxide at some time during the 3-hour experimental period. Experiments were therefore conducted in which the experimental period was varied, and rates of photosynthesis were determined at the end of 0.5, 1, 2, and 3 hours. When relatively light samples weighing less than 0.5 g. per liter were used, the rates appeared to be about the same whether the experimental period was 2 or 3 hours (table 3). However, when the rates during the second and the third hours were calculated, it was apparent that photosynthesis was somewhat lower during the third hour, about three-fourths of that during the second hour when sea water was used. A similar though smaller fall of less than 10 per cent was observed, when the water was supplemented with HCO_3^-. When heavier samples were used, the fall of photosynthesis in the third hour even with additional HCO_3^- was significantly great. The rate was only about one-third of that in the

second hour when sea water was not supplemented with HCO_3^-, and about three-fourths in the presence of additional HCO_3^-. It is evident, therefore, that the decline in the rate of photosynthesis in the third hour, greatly exaggerated by the use of relatively more plant material and by lower ΣCO_2 concentration, was caused by depletion of carbon dioxide.

The rate of photosynthesis during the first hour was consistently lower than that of the 2- and 3-hour experimental periods, irrespective of the ΣCO_2 content of the solution (table 3). In some experiments, the rates for the first and second half hour were also determined. It was found that photosynthesis during the first half hour was still lower than that during the first hour period. The rate during the second half hour, however, was higher than that during the first hour, and about the same as that during the second hour. This indicated the presence of an induction period of 20 to 30 minutes during which the rate of photosynthesis increased from zero to a constant value. Such an induction period of about 20-minute duration has been reported by Emerson and Green (1934) for the photosynthesis of *Gigartina*.

Our next problem concerns the form of carbon dioxide which should be present in abundance to effect maximal photosynthesis; in other words, whether the true limiting factor is the ΣCO_2 or $[HCO_3^-]$ or $[CO_2]$. It has been shown by Osterhout and Dorcas (1925) that little or no CO_2 enters normal cells of *Valonia* except in the form of undissociated molecules. This is in agreement with the fact that, in general, undissociated molecules pass through cell membranes much more readily than do their charged ions. If this is indeed true with *Gelidium*, the concentration of CO_2 in the sea water, rather than the HCO_3^- concentration or ΣCO_2, is the limiting factor in photosynthesis. If,

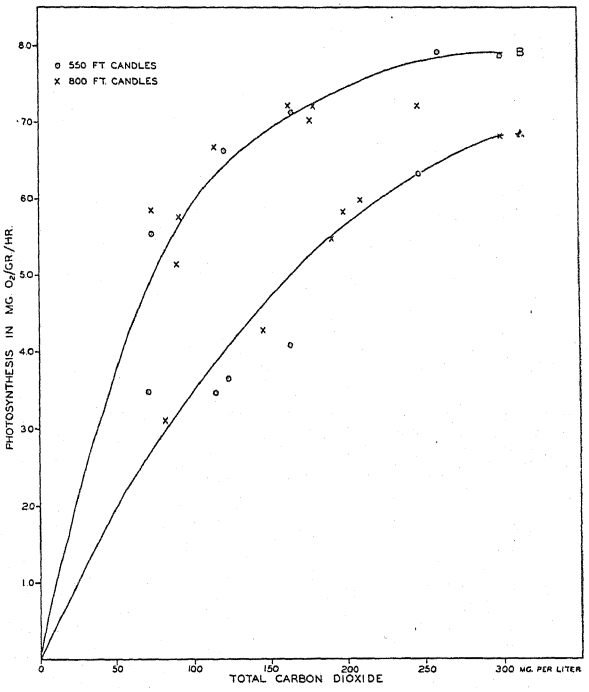


Fig. 1. The rate of photosynthesis of *Gelidium cartilagineum* in bicarbonate-supplemented sea water at 18.5 ± 0.5°C. in milligrams of oxygen per hour per gram of plant on the basis of oven-dried weight. Plants 0.8 to 0.9 g. per liter sea water in Curve A and 0.3 to 0.6 g. in Curve B. Each point represents photosynthesis of one plant sample during one 3-hour experimental period. Plants illuminated with "daylight" fluorescent lamps. Two light intensities employed: 500 foot-candles (⊙) and 800-foot-candles (×).

TABLE 3. *Rate of photosynthesis of Gelidium at different time intervals and under different treatments (from Experiments 46-4, 46-6 and 46-8).*

$[CO_2]$, moles × 10^{-6} per liter		Treatment	Dry weight in g. per liter	Hourly rates of photosynthesis in mg. O_2 per g. plant								
				For whole period				For each hour				
								1st hour			2nd	3rd
Initial	Final			½ hr.	1 hr.	2 hrs.	3 hrs.	1st ½	2nd ½	Whole	hr.	hr.
9	6	Filtered seawater	0.39–0.45	2.79	5.89	7.20	7.00	2.79	8.98	5.89	8.51	6.60
40	30	Seawater + $KHCO_3$	0.39–0.45	5.35	6.92	8.53	8.81	5.35	8.48	6.92	10.14	9.37
340	275	Seawater + HCl	0.23–0.45	5.17	7.66	9.03	10.26	5.17	10.14	7.66	10.37	12.72
360	322	Seawater + HCl + KH_2PO_4	0.21–0.35	6.57	8.90	11.31	10.33	6.57	11.22	8.90	13.72	8.37
4	2	Autoclaved seawater	1.27–1.75	...	4.96	4.61	3.67	...	...	4.96	4.26	1.79
4	2	Autoclaved seawater	0.77–1.13	...	5.07	5.97	4.68	...	...	5.07	6.87	2.10
6	4	Filtered seawater	0.62–0.81	...	4.15	5.48	4.62	...	...	4.15	6.81	2.90
23	18	Seawater + $KHCO_3$	0.63–0.92	...	7.56	7.42	6.80	...	...	7.56	7.28	5.56

upon this consideration, the data of figure 1 be replotted, using $[CO_2]$[5] rather than ΣCO_2 as abscissa, the higher points will be telescoped, and the apparent flattening in Curve B will disappear.

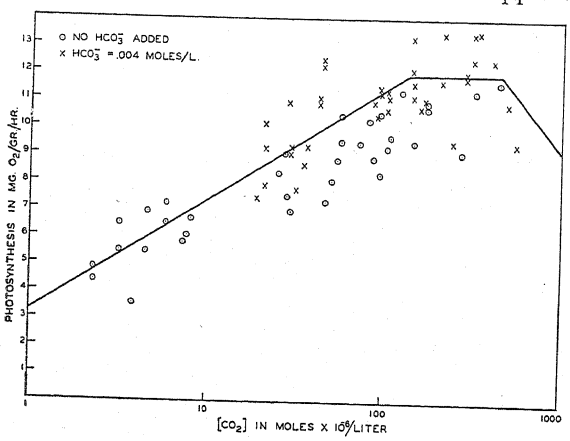


Fig. 2. The rate of photosynthesis of *Gelidium cartilagineum* in sea water containing different concentrations of free carbon dioxide (see text), with (×) and without (⊙) supplementary bicarbonate (0.004 M). Each point represents the photosynthesis of one plant sample during a 2-hour experimental period. Plants illuminated with "daylight" fluorescent lamps at an intensity of 780 foot-candles.

It is evident that the optimum carbon dioxide concentration had not been reached.

Experiments were therefore conducted in which the $[CO_2]$ was further increased above that in 0.005 M HCO_3^- sea water solutions by the addition of dilute HCl, with or without supplementary bicarbonate. As figure 2 shows, when $[CO_2]$ was thus increased, the rate of photosynthesis reached a high value of 10 to 13 mg. O_2 per gram *Gelidium* per hour (table 3). It will be noted that in this curve, photosynthesis increased with increasing CO_2, whether this CO_2 was derived from the bicarbonate added (×) or from that already present in sea water and set free by the addition of acid (⊙). The rate of photosynthesis was similar whether the water samples contained about 0.0015 M HCO_3^- but enough acid added to lower the pH to 7, or they contained 0.004 M HCO_3^- at pH 7.6. The addition of acid increased both $[CO_2]$ and $[HCO_3^-]$ as the pH was lowered to about pH 7.7. Below this pH, however, only the $[CO_2]$ increases with increasing hydrogen ion concentration (*cf.* Moberg *et al.*, 1934; p. 269; figure 6). It is therefore evident that $[CO_2]$ in the external medium, rather than the bicarbonate ion, determines the rate of photosynthesis. This is probably due to the inability of HCO_3^- to penetrate into the protoplasm of *Gelidium*, where photosynthesis takes place. Osterhout and Dorcas showed that the bicarbonate ion did not penetrate into the *cell sap* of *Valonia;* however, their conclusion does not necessarily apply to the *protoplasm*. The data, of course, give no indication of the actual role of HCO_3^- in photosynthesis, once inside the cell membrane; they apply only to the form in which CO_2 enters the cells.

Figure 2 also shows that the saturating $[CO_2]$ is between 100 and 150 × 10^{-6} mole per liter, which is considerably more than that ordinarily present in sea water. The spread of the points on this curve makes it difficult to determine the exact position of the optimum, which is more clearly shown in figure 3, Curve A. In this curve, the rate of photosynthesis is plotted against the pH, rather than against $[CO_2]$ of the sea water. Photosynthesis increases as the pH decreases until about pH 7.1. Beyond this pH, photosynthesis still keeps on increasing, though rather gradually, during the first experimental period (Curve A), but definitely shows a rapid decline during the third and fourth experimental period (Curve B), apparently due to acid injury. It thus appears that sea water at pH 7.1, having a $[CO_2]$ of 115 × 10^{-6} mole per liter, provides sufficient amount of CO_2 for maximal photosynthesis of *Gelidium* without causing acid injury. It is also clear that the rate of photosynthesis continues to increase with decreasing pH well below pH 7.7 while the bicarbonate concentration remains constant.

In figure 4, the rate of photosynthesis in bicarbonate-supplemented sea water, with total HCO_3^- concentration about 0.004 M, is plotted against pH. Since for any given pH the $[CO_2]$ is higher than the corresponding ones in figure 3, the curve is shifted to the right and a plateau is reached at

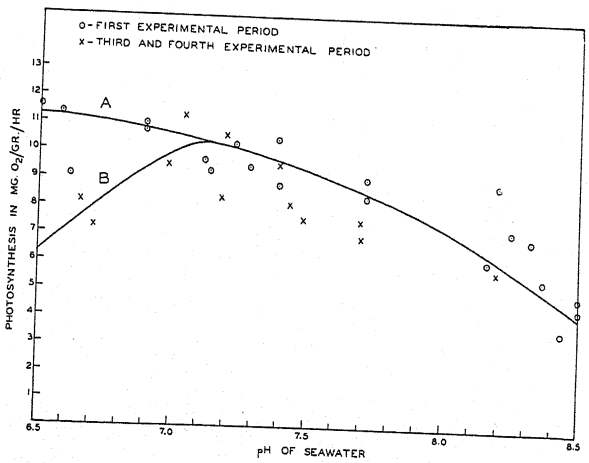


Fig. 3. The rate of photosynthesis of *Gelidium cartilagineum* in sea water at different pH values. Determinations in Curve A made during the first 2-hour period of an experiment (⊙), and in Curve B made during the third and fourth period (×). Rates for the second period fell between those for the first and the third and are not shown. Each point represents the photosynthesis of one plant sample during a 2-hour experimental period. Plants illuminated with "daylight" fluorescent lamps at an intensity of 780 foot-candles.

[5] In the experiments upon which this curve is based, only the original pH of the solutions was determined. Since there was a considerable shift in pH during the course of the experiment, calculations of CO_2 were of necessity approximate.

about pH 7.5. As in figure 3, however, the optimum $[CO_2]$ is still about 120×10^{-6} mole per liter. Thus, there is a close agreement in the lowest saturating $[CO_2]$ for maximum photosynthetic activity. Therefore, for the photosynthesis of *Gelidium,* the lower range of $[CO_2]$ giving maximum activity may be located at close to 120×10^{-6} mole per liter. The upper range has not been determined since the entry of acid injury (see below) as a factor complicated the matter. It can be said, however, that as high as 400×10^{-6} mole/l. CO_2 still yields optimum photosynthesis.

As table 3 shows, photosynthesis did not decline during the third hour of an experiment when the $[CO_2]$ was increased above the saturating concentration by the addition of HCl. When KH_2PO_4 was used instead, the rate of photosynthesis was as high or higher during the second hour, but decreased drastically in the third hour, evidently an indication of the toxicity of phosphate at the concentration used (0.004 M).

As mentioned earlier, Emerson and Green (1934) found the optimum $[CO_2]$ for the photosynthesis of *Gigartina harveyana* to be at about 400×10^{-6} mole per liter. The lower optimum for *Gelidium cartilagineum* may be correlated with its habitat in vigorously agitated water in which carbon dioxide is replenished more rapidly than in the quieter water where *Gigartina* grows. According to Rabinowitch (1945, p. 196), the saturation of the photosynthetic apparatus of *Chlorella,* a fresh-water alga, occurs at a relatively low carbon dioxide concentration of 50×10^{-6} mole per liter.

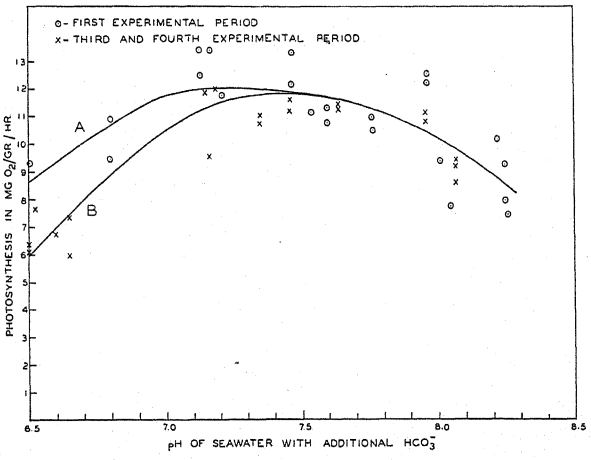


Fig. 4. The rate of photosynthesis of *Gelidium cartilagineum* in sea water supplemented with bicarbonate (0.004 M) at different pH values. Determinations in Curve A made during the first 2-hour period of an experiment (⊙), and in Curve B made during the third and fourth periods (×). Rates for the second period fell between those for the first and the third and are not shown. Each point represents the photosynthesis of one plant sample during a 2-hour experimental period. Plants illuminated with "daylight" fluorescent lamps at an intensity of 780 foot-candles.

The curves for the rate of photosynthesis at different pH values show evidence of acid injury below pH 7. This is apparent in sea water containing 0.004 M HCO_3^- even during the first two-hour

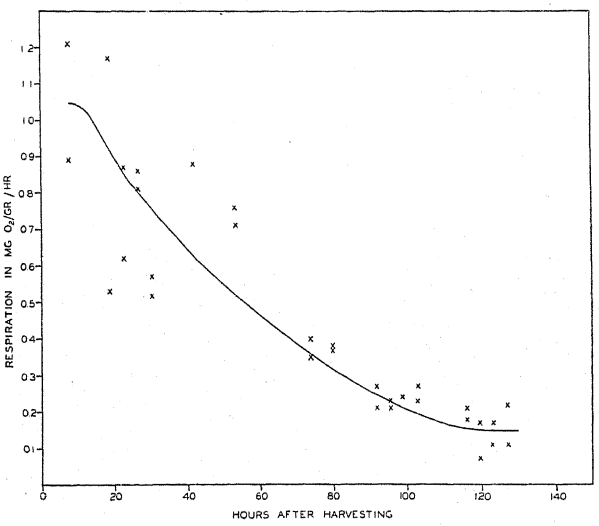


Fig. 5. The rate of respiration of *Gelidium cartilagineum* at different times after harvesting from the sea. Each point represents the respiration of one plant sample during a 3-hour experimental period at 18.5 ± 0.5°C.

period of an experiment, and becomes increasingly marked at the second, third, and fourth experimental periods during which the plants were exposed to low pH (Curves B, fig. 3, 4). That solutions containing 0.004 M HCO_3^-, but not those without added HCO_3^-, showed acid injury during the first two hours was probably due to the buffering capacity of the bicarbonate which prevented large shifts in the pH during the experimental period. Thus plants were exposed to a low pH for a longer time than those in unbuffered acidulated sea water, the pH of which increased much more rapidly. Above neutrality, there was no detectable injury. In one experiment, plants were exposed to solutions of different pH, as low as pH 7.1, for 24 hours; no changes in the rate of photosynthesis were observed. Acid injury evidently accounts for the fall in the rate of photosynthesis at very high $[CO_2]$, the pH of which was below 7 (fig. 2).

One of the objects of this paper was to devise an experimental technique which would avoid limitation of the rate of photosynthesis of *Gelidium* by carbon dioxide, so that the effect of other factors on photosynthesis could be studied. This is especially useful with ecological-physiological experiments in which the "bottle" methods are employed. On the basis of our experiments, we believe that the carbon dioxide as a variable factor in photosynthesis of *Gelidium cartilagineum* may be eliminated when the following conditions are observed: (1) the $[CO_2]$ is maintained above 120×10^{-6} mole per liter, (2) the pH is maintained above 7, and (3) the amount of plant used, on the basis of

dry weight, does not exceed 0.6 g. per liter sea water.

The following procedure will be used in our future experiments: sea water containing 0.004 M HCO^-_3 at pH 7.2 ($[CO_2]$ about 240×10^{-6} mole/l.) in bottles of 270 m.l. capacity containing pieces of *Gelidium* with a dry weight of about 100 mg.

RESPIRATION AND THE PHOTOSYNTHETIC QUOTIENT.—In the course of studies of the effect of carbon dioxide on photosynthesis of *Gelidium,* other information concerning its photosynthesis and respiration has been obtained. Photosynthetic quotients (P.Q.) and respiratory quotients (R.Q.) are of value in that they indicate the products of photosynthesis and the substrates of respiration. These were calculated in some experiments where $\Sigma\ CO_2$ as well as O_2 was determined at the end of each experimental period (table 2). Acid was not added to the sea water in these studies. The P.Q. $\left(\frac{+O_2}{-CO_2}\right)$ varied from 1.15 to 0.88 averaging *ca.* 0.95 ± 0.131[6], on the basis of 41 determinations, and the average value for R.Q. $\left(\frac{+CO_2}{-O_2}\right)$ was 1.11 ± 0.284[6]. Thus these values did not differ significantly from one, indicating that, as in other algae and in higher plants, carbohydrate is the photosynthetic product and the respiratory substrate. The determination of R.Q. was necessarily less accurate than that of P.Q. because of the smaller amount of gas exchange. For this reason, if apparent photosynthesis is used, instead of the values corrected for respiration, the resulting P.Q., 0.97 ± 0.017, is even closer to unity.

It was consistently found that the rate of respiration of *Gelidium* decreased with time after its collection (fig. 5). No parallel decrease was obtained, however, in the rate of apparent photosynthesis with the time of collection, although some experiments had run for as long as 4 to 5 days. It is probable that respiration of this algae in the first day or so after its collection represents the normal rate of respiration in the natural condition, since the amount of wounding in the preparation of the material for the experiment is very small. Very likely, the decline is due to the depletion of some unknown factors which is probably not a product of photosynthesis, since previous exposure to light did not affect the process.

COMPARISON OF PHOTOSYNTHESIS AND RESPIRATION OF GELIDIUM AND GIGARTINA.—It might be of some interest to compare the photosynthetic activity of *Gelidium cartilagineum* with that of *Gigartina harveyana.* Emerson and Green (1934) gave the rates of photosynthesis of *Gigartina* in terms of mg. of CO_2 consumed in 5 hours per 100 sq. cm. Since our data were in mg. of O_2 per g. dry weight of the plant per hour, we have recalculated Emerson and Green's data to make the rates directly comparable. For this purpose, we have obtained the area: weight ratio of *Gigartina* from specimens collected at Pacific Grove. The recalculation gave a value of about 20 mg. of O_2 per g. plant per hour at 15°C. The hourly yield of photosynthesis of *Gelidium* was about 12 mg. per g. plant at 18°C. under favorable carbon dioxide tension. Thus, weight for weight, disregarding the small temperature difference, the photosynthetic activity of *Gelidium* was about 60 per cent of that of *Gigartina.* This was expected, since the latter is a broad membranous form while the former is pinnately branched and consists of both leaf- and stem-like portions with considerably more photosynthetically inactive tissues.

Respiration of marine algae in general is supposed to be small. Emerson and Green (1934) found the respiration of *Gigartina harveyana* to be only about 1/30 of the maximum photosynthesis at 15°C. These authors did not mention whether there is a decline in the rate of respiration with time, as

[6] S. D. $= \pm\sqrt{\frac{\Sigma\ d^2}{n-1}}$.

TABLE 4. *Photosynthetic and respiratory quotients, from the data of table 2. In calculating the R.Q., the carbon dioxide produced was converted from mg./l. to mg./g./hr. according to the formula:* $R_2 = \frac{CV}{WT}$.

Plant	Photosynthetic quotient (P.Q.) $= \frac{+O_2}{-CO_2}$					Respiratory quotient (R.Q.) $= \frac{+CO_2}{-O_2}$				
	$+O_2$/g./hr.		$-CO^2$/g./hr.			$+CO^2$/g./hr.		$-O_2$/g./hr.		
	mg.	ml.	mg.	ml.	P.Q.	mg.	ml.	mg.	ml.	R.Q.
A	3.17	2.22	4.39	2.24	0.99	0.48	0.245	0.29	0.203	1.21
B	3.03	2.12	4.29	2.18	0.97	0.20	0.102	0.12	0.084	1.21
C	4.19	2.94	6.30	3.21	0.92	0.39	0.199	0.29	0.203	0.98
D	4.35	3.04	6.32	3.22	0.94	0.51	0.261	0.31	0.217	1.20
E	5.89	4.12	9.09	4.63	0.89	0.59	0.301	0.31	0.217	1.39
F	5.08	3.56	6.97	3.55	1.00	0.38	0.194	0.32	0.224	0.87
G	6.25	4.37	9.90	5.04	0.87	0.63	0.321	0.28	0.196	1.64
H	6.10	4.27	8.47	4.31	0.99	0.37	0.189	0.28	0.196	0.96
Average					0.95 ± 0.049					1.18 ± 0.24

we have found in *Gelidium*. The rate of respiration of *Gelidium* is about 1/10 of the maximum rate of photosynthesis at 18°C. during the first day after collection. It gradually declines during the following 4 to 5 days, and the ratio finally becomes stabilized at about 1/50. If the figure of Emerson and Green for *Gigartina* and ours for the initial respiration of *Gelidium* be taken as the respective normal rates under natural conditions, the respiratory activity of *Gelidium* is about three times that of *Gigartina*. This is probably true in view of the fact that *Gelidium cartilagineum* grows only in greatly agitated waters, whereas *Gigartina harveyana* lives in comparatively quieter waters. The high respiratory rate of the former possibly restricts its growth to places where the water is saturated with oxygen.

SUMMARY

Photosynthesis of *Gelidium cartilagineum* in sea water from La Jolla, California, was found to be limited by the carbon dioxide factor. It was shown that free carbon dioxide in the external media, rather than the bicarbonate ion, was the limiting factor. The saturating concentration of free carbon dioxide appeared to be above 110×10^{-6} mole/l. Effective increase of the free carbon dioxide concentration of sea water was best achieved by adding sodium or potassium bicarbonate and lowering the pH by means of an acid. There was, however, a marked acid injury to plants kept at a pH below 7 for any considerable time. When the amount of plant material used in an experiment exceeded 0.6 g. per liter of the solution, the rate of photosynthesis was retarded. On the basis of these findings, a method is outlined for the study of the effect of other factors on the photosynthesis of *Gelidium* in which carbon dioxide is eliminated as a limiting factor. Photosynthetic and respiratory quotients were found not to deviate significantly from unity. A decline was observed in the rate of respiration with time after collection of the material.

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HETEROSIS IN SORGHUM RESULTING FROM THE HETEROZYGOUS CONDITION OF A SINGLE GENE THAT AFFECTS DURATION OF GROWTH [1]

J. R. Quinby and R. E. Karper

THE LITERATURE bearing on theories of heterosis has been reviewed by Whaley (1944) with the exception of the early literature that had previously been thoroughly covered by Jones (1918). It is apparent from this review that the term heterosis has been applied variously in different cases and that no one theory has been demonstrated to cover all the facts. Complementary gene action, masking of deleterious recessives, combination of favorable dominants, and an effect peculiar to heterozygosity have all been shown to produce the effect. The theory of dominant interaction is apparently the theory most generally accepted by geneticists. Hull (1945) and Richey (1945) have recently based recommendations for isolating better foundation inbreds for use in corn hybrids on the assumption that hybrid vigor resides in the interaction of allelic genes and that numerous genes are involved.

Singleton (1943) has reported cases in *Zea Mays* L. where two parents differing in a single gene crossed to a third strain produce plants differing in vigor. Jones (1944, 1945) has recently reported cases in corn where plants heterozygous for a single allele are larger and higher yielding plants than the homozygous dominant, and the stimulus appears in these cases to be heterozygosity as originally postulated by East (1909), East and Hayes (1912), and Shull (1914). Randolph (1942), after observing the effects on growth from doubling the chromosome number of both inbred lines and hybrid strains of corn, came to the conclusion that heterozygosity in itself may be responsible for much of the vigor or heterosis exhibited by hybrids. Jones (1945) has pointed out that only certain genes in the heterozygous condition produce heterosis and that heterogosity in itself does not produce the effect.

In view of the complexity of the problem and of the practical importance of knowing what the causes of heterosis are, any information that has a bearing upon any of the presently held theories would be welcome.

The inheritance of three genes that influence duration of growth in milo, a group of *Sorghum vulgare* Pers. has been reported by Quinby and Karper (1945). Lateness is dominant to earliness except that recessive *ma* is epistatic to dominant Ma_2 and Ma_3, and Ma_2 is epistatic to Ma_3. This state of affairs results in eight homozygous genotypes but only four phenotypes for maturity that have been called Early (*ma* Ma_2 Ma_3, *ma* ma_2 Ma_3, *ma* Ma_2 ma_3, *ma* ma_2 ma_3), Intermediate (*Ma* ma_2 ma_3), Late (*Ma* ma_2 Ma_3) and Ultra-late (*Ma* Ma_2 ma_3, *Ma* Ma_2 Ma_3). These phenotypes bloom at approximately 50, 70, 82, and 98 days if planted in June at Chillicothe, Texas, when the days are longer than 14 hours. All four phenotypes for maturity are identical for both size and duration of growth when grown under 10-hour photoperiods. The genes, therefore, govern the response of milo to photoperiod. Since these genes control the time of floral initiation and floral initiation is now generally thought to be stimulated by some hormone-like substance as postulated by Hamner (1942), it seems reasonable to believe that these three genes in milo in some way influence the synthesis of such a substance.

During the study of the inheritance of the maturity of milo it became apparent that the F_2 genotypes heterozygous for the first gene (*Ma ma*) were different in maturity and size from the homozygous dominants (*Ma Ma*) which they might have been expected to resemble in view of the prevailing dominance. The time of anthesis of various homozygous and heterozygous genotypes is shown in table 1. The figures given for date of anthesis are not the means of any given population but are figures typical of the data that have accumulated over a period of several years. There is relatively little variation from year to year in the time of anthesis of the Early and Intermediate genotypes but the strains of later maturity that are not adapted agronomically are subject to such vagaries of weather that it is not unusual for any one of the three later maturing genotypes to vary in time of anthesis by as much as 15 days from one year to the next. On this account the figures in table 1 are more typical

TABLE 1. *The number of days from planting to anthesis of various homozygous and heterozygous genotypes of milo.*

Genotype		Number of days to anthesis
Early	$mamama_2ma_2ma_3ma_3$	50
Intermediate	$MaMama_2ma_2ma_3ma_3$	70
Intermediate-heterozygous	$Mamama_2ma_2ma_3ma_3$	82
Late	$MaMama_2ma_2Ma_3Ma_3$	82
Late-heterozygous	$Mamama_2ma_2Ma_3Ma_3$	92
Ultra-late	$MaMaMa_2Ma_2ma_3ma_3$	98
Ultra-late-heterozygous	$MamaMa_2Ma_2ma_3ma_3$	94
Ultra-late, 2	$MaMaMa_2Ma_2Ma_3Ma_3$	98
Ultra-late-heterozygous	$MamaMa_2Ma_2Ma_3Ma_3$	94

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TABLE 2. *Data on plant characters and yield of homozygous and heterozygous genotypes of milo.*

Genotype		Number of days to anthesis	Number of stalks	Head weight, grams	Stover weight, grams
		Planted June 20, 1944			
Early	$mamama_2ma_2ma_3ma_3$	51.2 ± .3	2.01 ± .09	93.6 ± 4.8	83.4 ± 5.3
Intermediate	$MaMama_2ma_2ma_3ma_3$	70.2 ± .5	2.06 ± .08	129.9 ± 6.2	146.0 ± 7.1
Intermediate-heterozygous	$Mamama_2ma_2ma_3ma_3$	83.2 ± .3	2.21 ± .06	148.9 ± 4.6	174.4 ± 5.6
		Planted June 9, 1942			
Early	$mamaMa_2Ma_2ma_3ma_3$	49.8 ± .5	1.20 ± .26	90.7 ± 18.1	
Ultra-late	$MaMaMa_2Ma_2ma_3ma_3$	95.5 ± .4	1.73 ± .13	149.7 ± 18.1	
Ultra-late-heterozygous	$MamaMa_2Ma_2ma_3ma_3$	93.3 ± .5	2.70 ± .20	240.4 ± 18.1	

than if means in some single year were used. Intermediate-heterozygous and Late-heterozygous genotypes are later in blooming than their corresponding homozygous genotypes. However, both of the Ultra-late-heterozygous genotypes are earlier in blooming than their corresponding homozygous genotypes.

The milo crop used in this study has been described and discussed fully in papers by Quinby and Karper (1945) and Karper and Quinby (1946). Varieties of sorghum are naturally inbred lines that are not changed by further inbreeding. The various genotypes of milo, although phenotypically distinct under normal growing conditions, are identical in size and maturity if grown under 10-hour photoperiods. There seems every reason to believe that the various genotypes of milo that were used as parents differ only in the genes that influence time of floral initiation.

There is correlation between time of maturity in sorghum and the size ultimately attained which is discussed by Quinby and Karper (1945). In homozygous genotypes, the difference in the size of plants is apparently the result of longer duration of growth without an indication of a difference in rate of growth as shown by data so far obtained. Earliness of maturity which is caused by early floral initiation is associated with small amount of growth and lateness with a large amount. If conditions remain favorable throughout the entire growing season, the yield of both grain and stover will increase progressively from early to late maturity. It usually happens, however, that a shortage of moisture reduces the grain yield of Late and Ultra-late genotypes which accounts for the fact that the Intermediate and Early genotypes are recommended for agricultural use. In heterozygous as compared to homozygous genotypes, duration of growth is still a factor of importance in determining size. However, there are indications that there are other effects of heterozygosity on growth and development.

In 1944, 474 plants in ten F_3 progeny rows of a cross between Dwarf Yellow milo (Intermediate, *Ma* ma_2 ma_3) × Sooner milo (Early, *ma* ma_2 ma_3) that were segregating for Early and Intermediate maturity were harvested and the air-dry weight of head and stover determined for each plant. The plants had previously been tagged for time of anthesis and the number of stalks comprising each plant was recorded at the time of harvest. These 474 plants were grown into the F_4 generation to identify the genetic constitution of each plant. The genotype of each plant is known, therefore, as well as its time of anthesis, number of stalks, and yield of both head and stover. The data are presented in table 2. The F_4 identification showed that there were 120 Early, 118 Intermediate, and 236 Intermediate-heterozygous plants which is an almost perfect fit to a 1:1:2 ratio expected from the segregation of a single allele. There was no appreciable variation among the F_4 rows of each genotype. Since the populations were all of the same kind, the data were all combined and the 474 plants treated as one population. The figures show that the Intermediate-heterozygous genotype bloomed 13 days later than the homozygous Intermediate genotype, had 7 per cent more stalks per plant, and produced 14 per cent more heads and 19 per cent more stover. The differences between the two genotypes are all statistically significant except that of number of stalks. When the average numbers of stalks of Intermediate and Intermediate-heterozygous genotypes of each of the ten families were paired, the difference in tillering between the two genotypes was also found to be statistically significant. The Standard Error of the difference of 0.1552 stalks was 0.0602 which yielded a t of 2.577 with nine degrees of freedom.

Such increases, with the exception of the increase in tillering, would be expected in view of the larger plant produced as the result of delayed floral initiation of heterozygous plants if the growing season had been favorable. However, growing conditions became progressively more unfavorable as the season advanced and the Late genotype in a planting on the same date as that of the ten heterozygous progenies, but more favorably located as to soil fertility, produced yields of both grain and stover not significantly different from those of the Intermediate genotype. The data which were obtained from 40 plants of each genotype are presented in

TABLE 3. *Days to anthesis and yield of heads and forage of Intermediate and Late genotypes of milo planted on June 20, 1944.*

Genotype		Number days to anthesis	Head weight, grams	Forage weight, grams
Intermediate	Ma Ma ma_2 ma_2 ma_3 ma_3	68.6	163.3 ± 9.1	281.2 ± 18.1
Late	Ma Ma ma_2 ma_2 Ma_3 Ma_3	82.6	149.7 ± 13.6	290.3 ± 18.1

table 3. The Late genotype occupied a growing period almost identical to that of the Intermediate-heterozygous genotype yet it produced a yield of heads and forage not significantly different from that of the homozygous Intermediate genotype, whereas the Intermediate-heterozygous genotype produced more heads and stover than the Intermediate genotype by 14 and 19 per cent respectively. A difference in rate of growth in favor of the Intermediate-heterozygous over the Late genotype is indicated even though the two genotypes were not compared directly.

The only comparative yield so far obtained on a heterozygous genotype of milo other than Intermediate-heterozygous was obtained in 1942 when an F_3 progeny row segregating for Early (*ma* Ma_2 ma_3) and Ultra-late (Ma Ma_2 ma_3) was harvested. The data were obtained from 44 plants and when identified a year later, 10 were found to be Early (*ma ma* Ma_2 Ma_2 ma_3 ma_3), 11 were Ultra-late (Ma Ma Ma_2 Ma_2 ma_3 ma_3), and 23 were Ultra-late-heterozygous (Ma *ma* Ma_2 Ma_2 ma_3 ma_3). These figures are a close fit to a 1:1:2 ratio expected from the segregation of a single allele. Stover yields were not obtained from this population. The Ultra-late-heterozygous plants produced statistically significant increases of 56 per cent in stalks and 63 per cent in weight of heads per plant above homozygous Ultra-late plants. The data are given in table 2. Since tiller heads are smaller than main heads, an increase of 56 per cent in stalks would not produce a 63 per cent increase in weight of heads. The indications are, therefore, that the main heads of Ultra-late-heterozygous plants were larger than those of Ultra-late plants even though they were produced in a shorter time.

TABLE 4. *Data showing amount of tillering and grain yield of parents and a first generation hybrid of kafir.*

	Stalks per plant	Yield of grain per plant, gms.
Planted June 8, 1933		
Parents:		
Blackhul kafir	1.03 ± .11	90.7 ± 3.2
Red kafir	1.03 ± .11	59.0 ± 4.1
Hybrid:		
Blackhul kafir × Red kafir	1.70 ± .15	195.0 ± 13.5

It is apparent that more tillering must be one of the manifestations of heterosis in sorghum and data published in an earlier paper by Karper and Quinby (1937) and given again in table 4 supports the hypothesis. In 1933 an F_1 population of a cross between strains of Red kafir and Blackhul kafir, two non-tillering types, and the two parents were grown. Each population consisted of 30 plants. The hybrid which had the same maturity as each of the parents produced 65 per cent more stalks and more than twice as much grain as the better producing parent. The differences were statistically significant. It is not known of course in how many genes these two strains of kafir differ but no pure breeding, tillering type could be found in later generations. Also, no strain higher yielding than Blackhul kafir was selected from the progeny of the cross.

DISCUSSION.—There are no data presented in this paper to show that heterozygous sorghum plants have a greater growth rate than homozygous plants. It is shown, however, that heterozygous sorghum plants are larger than homozygous plants of comparable growth duration. A difference in growth rate must exist in *Sorghum vulgare* Pers., therefore, and is known to exist in *Zea Mays* L. It is obvious that a hybrid plant that reaches maturity ahead of its inbred parents and attains a much greater size must, at some time, have grown at a faster rate. Ashby (1930) and Sprague (1936) have shown a lack of differential growth between inbreds and hybrids of corn during the grand period of growth which consists primarily in cell enlargement. East and Hayes (1912) in reporting some of the early work on the causes of hybrid vigor stated that "the vigor due to heterozygosis is primarily an increase and an acceleration of cell division; in other words, increased power of assimilation." Kiesselbach (1922) found that the major part of the expression of heterosis in corn hybrids resulted from a difference in number of cells rather than size of cells. Since a difference in number of cells distinguishes the inbreds and hybrids, a difference in rate of growth of the meristem is indicated. Such a conclusion is in conformity with those of Sprague (1936), Kempton and McLane (1942), and Bernstein (1943) that a period of differential growth occurs during the embryonic and seedling stages. Ashby (1930) reported a difference in embryonic growth but failed to properly sample his material during the period of meristematic growth following germination. On this account, he failed to recognize the faster rate of seedling growth exhibited by hybrids later observed by Sprague, Kempton and McLane, and Bernstein.

Tillering is shown here to be a manifestation of heterosis in sorghum. Singleton and Nelson (1945) have shown that a reduction in the amount of tillering in sweet corn accompanies inbreeding. Tiller initiation takes place in sorghum before floral differentiation and during the time of meristematic growth. A greater rate of cell division and greater tillering of sorghum hybrids are evidently caused by a stimulation that results in hybrid vigor. In the case of the *Ma* gene in sorghum, the heterozygous condition of this one gene results in heterosis. The cause of the stimulation in meristematic growth is not explained but must grow out of some biochemical reaction that is different in homozygous and heterozygous individuals.

Beadle and Tatum (1941) and Horowitz *et al.* (1945) have developed the thesis that genes act by determining the specificities of enzyme proteins and as a consequence control specific chemical reactions; and, according to Mitchell and Houlahan (1946), a gene is normally concerned with a step in the sequence of reactions leading to the production of an essential growth substance. They consider that the gene controls the production of a specific enzyme that in turn catalyzes a specific reaction. Robbins (1940) has found that an unidentified growth substance or coenzyme that he has designated as factor Z is present in greater amounts in hybrid than in inbred endosperms and embryos of corn seeds. Bernstein (1943) thought it likely that the earlier digestion of starch and accumulation of reducing sugars in hybrid than in inbred endosperms of corn that followed more rapid water uptake by hybrids was caused by a greater concentration of factor Z. This sequence of events, caused by a greater concentration of factor Z, was considered by Bernstein as the probable explanation of a greater growth rate exhibited by young hybrid corn plants.

Thimann and Skoog (1933, 1934) and Went and Thimann (1937) have reported that the concentration of auxin influences tillering. Since tillering is one of the manifestations of heterosis, this is additional evidence that hormones or vitamin-like growth substances play a part in producing heterosis. Avery (1939) has suggested that effects produced by auxin may account for heterosis. Pratt and Albaum (1939) have found in sorghum that a reduction in auxin content brought about by immersing germinating seeds in water reduced shoot growth of the seedlings. The concentration of auxin is known, therefore, to influence shoot growth of sorghum seedlings. Furthermore, the same authors showed that kafir seeds that started out with an amount of auxin equal to milo seeds had, after the experimental treatment, considerably more hormone than milo seeds. They pointed out that one of the three factors that may be involved is the rate of auxin formation. It may be that milo is a tillering variety because of a low rate and kafir a non-tillering variety because of a high rate of auxin production. Thimann and Lane (1938) have suggested tentatively that the vegetative effects of vernalization are due to the prolonged exposure of the seed to its internal auxin supply. The *Ma* gene in sorghum is one that is influenced by photoperiod and one that might be expected to influence the synthesis of auxin and affect the internal auxin supply.

The cause of the stimulation resulting from heterozygosity that results in hybrid vigor is not explained by saying that a difference in coenzyme or hormone synthesis or activity must exist in homozygous and heterozygous individuals. Robbins (1942–1943) is of the opinion that the amount or activity of one or more enzyme-systems as determined by the quantity of its necessary associate, a coenzyme, act as internal factors limiting growth; and that, in the case of partial deficiencies, the effect is to decrease the rate of growth but not to inhibit it entirely. In sorghum, it may be that the dominant gene, *Ma,* and recessive, *ma,* are members of an allelic series each member of which causes a partial deficiency of some substance that catalyzes the reactions that bring about growth; and that, in the heterozygous condition, part or all of the deficiency is satisfied. Such a condition would result in homozygotes that are unable to make full use of the nutrients available to them, and in heterozygotes that are able to make greater use of the same nutrient supply.

Since the heterozygous condition of a single gene results in heterosis, there is support for the theory that an interaction between unlike allelomorphs causes heterosis. It is conceivable that in most cases of hybrid vigor, the heterozygous condition of more than one gene is involved. However, the heterozygous condition of a single gene, *Ma ma,* in sorghum results in hybrid vigor of a magnitude comparable to that shown by commercial varieties of hybrid corn. Singleton (1943) and Jones (1944, 1945) have, in corn, observed hybrid vigor resulting from the heterozygous condition of a single gene, but Singleton and Nelson (1945) consider these cases as special, rather than general, manifestations of hybrid vigor. The probability is that these cases are typical rather than unique.

The data show that other genes may contribute to the magnitude of hybrid vigor without themselves causing heterosis. The homozygous dominant condition of the second and third genes, $Ma_2\ Ma_2$ and $Ma_3\ Ma_3$, both contribute to a delay in floral initiation. When the stimulus to greater meristematic growth is contributed by the heterozygous condition of the *Ma ma* gene, a delay in floral initiation produced by either dominant $Ma_2\ Ma_2$ or $Ma_3\ Ma_3$ allows the stimulus to act over a longer time as the shoot axis is meristematic longer. This stimulation acting over a longer period will result in a greater expression of hybrid vigor in $Ma_2\ Ma_2$ and $Ma_3\ Ma_3$ genotypes even though these genes contribute nothing in the way of a stimulus to growth. Superficially, this contribution to the expression of heterosis of non-allelomorphs acting on heterozygous

Ma ma appears to be a case of interaction between non-allelic genes.

The genes reported by Jones (1945) and Singleton (1943) as producing vigor in the heterozygous condition are more or less deleterious which could hardly be said to be true of the *ma* gene in sorghum since no abnormality is produced. Recessive *ma* does, however, reduce the size of plant. In common with the genes in corn, however, this gene in sorghum does affect some fundamental growth process and one might even be justified in suspecting that many genes that affect organ size or are quantitative rather than qualitative will be found to have interactions that are different in the homozygous and heterozygous conditions.

The results reported here with sorghum throw doubt upon the necessity of assuming the interaction of numerous genes in an F_1 hybrid to bring about hybrid vigor of the magnitude generally observed. The greater rate of cell division in the meristem of both corn and sorghum that results in hybrid vigor is due to heterosis in the exact sense in which Shull (1914) proposed the use of the word "heterosis."

SUMMARY

There is a gene *Ma* in sorghum that controls plant response to photoperiod and influences time of floral initiation. This gene in the heterozygous condition, *Ma ma,* produces plants that differ in maturity from homozygous genotypes. In addition, the heterozygous condition of this gene produces plants that are larger and tiller more than homozygous genotypes of comparable growth duration.

The conclusion is that heterosis in sorghum is a stimulation to tillering and to cell division. Homozygotes are conceived as being unable to make full use of the nutrients available to them whereas heterozygotes are able to make greater use of the same nutrient supply.

When the stimulus to greater meristematic growth is contributed by the heterozygous condition of the *Ma ma* gene, dominant genes Ma_2 Ma_2 and Ma_3 Ma_3, which delay floral initiation and maturity, allow the stimulus to act over a longer period of time. This condition results in a greater expression of hybrid vigor in Ma_2 Ma_2 and Ma_3 Ma_3 genotypes even though these genes contribute nothing in the way of a stimulus to meristematic growth.

The heterozygous condition of the single gene, *Ma ma,* in sorghum results in hybrid vigor of a magnitude comparable to that observed in commercial varieties of hybrid corn.

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RELATIONS BETWEEN TISSUE ORGANIZATION AND VASCULARIZATION IN LEAVES OF CERTAIN TROPICAL AND SUBTROPICAL DICOTYLEDONS [1]

Robert B. Wylie

THIS PAPER takes up further consideration of relations between the nonvascular tissues of the blade and its venation. The study included leaves from ninety of the tropical and subtropical dicotyledons growing in southern Florida. About two-thirds of these were introduced plants from widely scattered areas in the tropics.

Foliage leaves are the plant's most exposed and least protected vegetative organs and they usually possess an elaborate venation. Reasons for this seeming overvascularization are suggested by their form, structure and functions. The flattened blade involves a wider spread of conductive channels and increased external surface in comparison with a radial structure of equal volume. The great area of moist cell wall exposed upon intercellular space markedly increases the transpiration loss. Leaves have limited provision for water storage and are subjected to sudden environmental changes. Their water relations are further complicated by the necessarily restricted coverings through which light reaches the mesophyll. Protection of the blade is limited primarily to a film of cuticle over outer epidermal cell walls; leaves are thus denied the advantages of cork and bark so freely used by stems and roots. Perhaps the most critical aspect of distribution is related to the slower movement of materials through the living cells lying between vascular channels. These are some of the factors directly or indirectly related to the venation of the leaf.

The average distance between minor veins for twenty-two Iowa deciduous trees and shrubs was 112 μ measured from xylem to xylem (Wylie, 1939); subtracting the thickness of the border parenchyma left only 90 μ for the spread of mesophyll between vascular strands. These blades had a mean thickness of 152 μ, so, fully 85 per cent of their living cells were within 75 μ of a vein and most of the mesophyll was much nearer to vascular supply. Measurements by Plymale and Wylie (1944) for five of these species showed that their venation and associated tissues, omitting all protruding portions of larger veins, occupied one-fourth of the blade volume between epidermal layers and so displaced 25 per cent of the potential chlorenchyma.

Experiments with living attached leaves have revealed that their veins possess an unexpected capacity for conduction of water. A single major vein (Plymale and Wylie, 1944) or a few minor veins (Wylie, 1938) might sustain the distal portion of a wounded blade for the remainder of the summer (two months or more) with little or no loss of tissue. There is increasing evidence that the venation in leaves of dicotyledons has a capacity for water conduction greatly in excess of normal need or even emergency demand. This suggests that factors other than conductive capacity also influence the vein-spacing in their blades.

Marked differences in venation may occur within a given species or even among leaves on the same plant. Schuster's work (1908) early directed attention to the differences in venation; comparing sun and shade leaves he found that the total vein length per unit area was usually much greater in sun leaves. Zalenski (1902) discovered numerous differences between leaves of higher and lower insertion on the same axis; the upper leaves usually had greater vascularization and this was generally associated with increased proportions of palisade. Benedict (1915) and Tellefsen (1922) held that senescence was responsible for the greater vein development which they reported for leaves of certain older trees and vines compared with that in younger plants of the same species. Less generally known are the considerable variations among different areas of the same blade. Some of these are discussed in my recent paper on *Hedera helix* L. (Wylie, 1944).

Whatever the more remote causes, the writer has shown for leaves of a number of dicotyledons, that vascularization is related to the organization of the non-vascular tissues of the blade. For leaves other than succulents or extreme xeromorphic types, veins are generally farther apart in blades having greater proportion of tissue with cells closely united and often elongated or expanded in the plane of the blade (sponge and epidermal layers). On the other hand those of similar

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thickness but having greater amount of palisade usually have veins closer together. While other factors are involved, the relative proportions of these two complementary sets of blade tissue are often closely associated with vein separation. This interpretation was supported by a survey of leaves from three groups of dicotyledons that differed in habit or habitat or both (Wylie, 1939). The coefficient of correlation between tissue-organization and vein-spacing for twenty-two Iowa trees and shrubs was 0.735 ± .059, for twenty-four Iowa herbaceous dicotyledons was 0.648 ± .08 and for twenty Pacific coast broad leaved evergreens was 0.684±08. While the number of species included in these groups was too small for broader generalizations, the results seem significant. The present paper outlines a similar survey of leaves from ninety tropical and subtropical species collected in southern Florida during the summer of 1944. The writer is under great obligation to members of the botany staff at the University of Miami for assistance in the collection and identification of this material, and especially to Curator W. M. Buswell who kindly verified determinations.

MATERIAL AND METHODS.—The Florida collections included leaves of 144 dicotyledons. Most of them were collected in the Miami area but scattered numbers were from as far south as Key West. Fully two-thirds were introduced woody plants which were mostly evergreen. Most of the native species collected were also woody but some of them were deciduous. Consistent effort was made to secure leaves normally differing in size, form, texture, and relationship rather than to seek material representative of any foliar types or taxonomic groups.

In addition to pressed specimens, portions of representative leaves of each species were killed for sectioning. These sample areas, cut into small rectangles before fixation in FAA, were subsequently treated with approximately 15 per cent hydrofluoric acid for a week before imbedding. With larger leaves the samples were taken from a median lateral area and for smaller blades the central region was used but no measurements were subsequently recorded from any tissues near a larger vein. Serial sections were cut 12 μ thick in both transverse and paradermal planes; the latter sectioned in the plane of the blade, were especially helpful in determining tissue relations. Delafield's haematoxylin in combination with safranin gave favorable differentiation of tissues for these purposes.

Cross sections were used to measure leaf thickness and to determine the volumes of the several blade tissues, other than veins. Paradermal sections permitted direct measurement of vein-spacing, or intervascular-intervals, and showed favorably the size, form, and arrangement of cells for all tissues viewed at right angles to the blade. Measurements of vein spacing were from xylem to xylem, so records included twice the radial thickness of the border parenchyma.

RESULTS.—A general survey of the sections showed several basic patterns of leaf organization among these leaves as well as marked differences in the amount and structure of all tissues. The present paper is limited to ninety species having favorable development of both spongy and palisade parenchyma. This excludes, at this time, many leaves that were predominantly palisade as well as certain other foliar types which will be taken up subsequently. A few were unfavorable for satisfactory paradermal sections.

The introduced species in general had much larger leaves than the native plants. Most of both groups were xeromorphic with some hammock plants showing mesomorphic tendencies. Many leaves were compound and among those with entire blades were diversified patterns of major venation. In brief, the leaves of this series constituted a heterogeneous assemblage of diverse taxonomic relationships.

In the survey of a given leaf its various blade tissues lying between veins were measured and classified into two divisions, based upon the form, degree of contact and predominant trend of their cells. One set, favoring transfer between veins, included all tissues having cells closely united laterally and often more or less extended in the plane of the blade; in this group were placed the sponge, epidermal layers and any subdermal zones having cells similarly disposed. The other division consisted chiefly of the palisade but also included any transitional layers with limited lateral contacts between their cells. With the former as numerator and the latter as denominator the relative volumes of these two sets of tissue yielded a ratio hereinafter referred to as SEE/P. In the large, this ratio, usually written as a decimal, represents for that leaf the relative proportions of its blade tissues having greater integration in either the horizontal or the vertical plane and consequently either more or less favorable for transfer between veins.

The well known differences between palisade and sponge need no comment but their contrasts, as seen in paradermal sections of a sample area from a blade of *Calophyllum calaba* Jacq., are illustrated by fig. 1. In this figure the cells of both are drawn to common scale and the contrasting possibilities of these tissues for conduction are apparent. Movement through the palisade in any direction in the plane of the blade would involve at least ten times as many cells as transport through an equal distance in like direction via the spongy mesophyll. The latter tissue is also favorably organized for transfer of materials to or from contiguous layers above or below.

For reasons to be discussed later vein-spacing rather than total vein length per unit area was used in evaluating the vascularization of these Florida leaves. My preliminary paper (Wylie, 1939), using this method, suggested its possibilities, and paradermal sections are peculiarly favorable for measurement of intervascular intervals. For each leaf

an attempt was made to establish the normal separation of its smaller veins. No larger veins were included in these measurements and neither the close approach of lesser veiens as near forks nor the position of vein tips in the mesophyll was considered significant because these are not expressions of normal spacing of minor veins, which make up over four-fifths of the total vein length of these leaves and which are in closest association with the mesophyll.

Because the pattern of the minor venation varies greatly there should be brief mention of the method used in approximating the intervascular interval. For a few of these species in which the ultimate veinlets are united around areas of mesophyll, twenty-five measurements of the least cross dimension of these polygons yielded a mean which was recorded as the IVI, or intervascular interval, for that leaf. But in most leaves having such meshes, small lateral veins, often branched, had penetrated into these areas, thus greatly reducing the intervascular distance. In many others the boundary veins were far apart, with an elaborate minor venation between; a large number of species had freely branching veins spread out between secondary major veins and were without meshes. Usually thirty to fifty measurements of spaces between minor veins gave a significant mean for that leaf sample. When the pattern was peculiarly complicated a larger number of measurements was made. Resurveys were often used as checks against earlier findings for a given species. In ten leaves, for which the intervascular interval was also checked against total vein length, the results were commensurate.

The largest leaf measured was that of *Firmiana platanifolia* (L.) R. Br.; the blade of a fallen leaf was over four square feet in area; at the opposite extreme were leaves or leaflets less than a square centimeter in size. Blade thickness for the leaves of this series ranged from 403 μ for *Calophyllum calaba* Jacq. to 98 μ for *Pithecolobium dulce* (Roxb.) Benth. Epidermal thickness for leaves having a single layer of epidermis ranged from 10 μ to 54 μ for the upper layer and from 6 μ to 30 μ for the lower epidermis. Total epidermal thickness averaged about 15 per cent greater for the native species as compared with the introduced dicotyledon leaves. Relatively few leaves had double epidermis and only one in ten had any special subdermal development of water storage tissue. The ratio between the two categories of non-vascular tissue was higher in the native species (1.44) as compared with the introduced dicotyledons (1.30). This difference was largely accounted for by the thicker epidermis of the native species. Most dicotyledon leaves have a transitional layer between the lower epidermis and the mesophyll which in the majority of these was so modified as to be classified with the palisade.

The completed record for each leaf included: (1) the mean *total thickness* of blade, (2) the *intervascular interval* (IVI) and (3) the value derived from SEE/P, representing the volumetric ratio of the two sets of non-vascular tissues in that blade. To facilitate comparisons, after the survey was completed the species were grouped according to their vein-spacing, and the several classes were arbitrarily separated by 20 μ increments in the intervascular interval. Finally, for the several species falling in each group, the means of corresponding values were determined and recorded in tabular form (table 1).

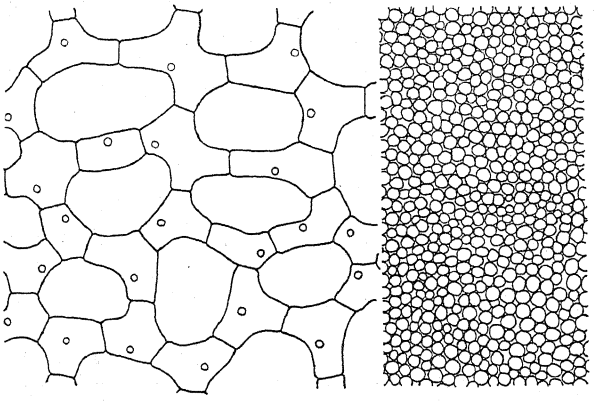


Fig. 1. Connected cells of spongy parenchyma and of palisade, from leaf of *Calophyllum calaba* Jacq. drawn to common scale from paradermal sections of the same leaf area (×270).

It is apparent from this table that there is a significant relation between columns IVI and SEE/P, since the values for both increase consistently through the groups. This tabulation also shows a degree of agreement between IVI and *blade thickness,* but this relation is not uniform and varies considerably among the several groups. It may be noted also that fifty-three species have an intervascular interval between 60 μ and 140 μ and that more than

TABLE 1. *Summary for leaves of Florida dicotyledons. The 90 species (33 native and 57 introduced) classified by groups according to IVI (intervascular interval), with 20 μ between classes.*[a]

IVI class (microns)	Number of spp.	Mean thick. (microns)	Mean IVI (microns)	Mean SEE/P[b] value
48–60	6	160	55.83	0.545
61–80	10	162	68.40	0.776
81–100	10	218	92.00	0.941
101–120	17	196	109.7	1.234
121–140	16	214	128.0	1.267
141–160	8	230	150.0	1.547
161–180	7	204	153.5	1.707
181–200	6	183	192.8	2.033
201–220	3	216	202.3	1.940
221–240	4	329	233.2	2.595
241–up	3	269	270.0	2.736

[a] The values here recorded for each class are the means for all species falling in that class.

[b] SEE/P represents the ratio between the volumes of tissues in the sponge and palisade categories.

TABLE 2. *Statistical analysis of data relating to leaves of 90 species of dicotyledons collected in southern Florida.*

Groups of spp. surveyed	Number of spp.	Mean blade thickness	Mean IVI value	Coefficients of correlation[a] between IVI and SEE/P	between IVI and thickness
Introduced species	57	202 μ	127.6 μ	0.8825 $\pm$ .02	0.3762 $\pm$ .08
Native Florida	33	210 μ	135.2 μ	0.9122 $\pm$ .02	0.2320 $\pm$ .11
Combined Florida	90	205 μ	130.4 μ	0.8855 $\pm$ .02	0.3298 $\pm$ .06

[a] Correlations were based upon analysis of all data for each group.

a third of them lie between 100 μ and 140 μ. The mean IVI for the entire series was found to be 130.4 μ.

Subsequently, statistical analysis of all data, by the Statistical Service of the State University of Iowa, revealed that the coefficient of correlation between leaf organization (SEE/P) and vein spacing (IVI) was 0.8855 $\pm$ .02, while that between blade thickness and IVI was 0.3298 $\pm$ .06 (table 2). This summary gives also the corresponding values, computed independently, for the groups of native Florida and introduced species.

The degree of correlation thus revealed between tissue organization and vein spacing is statistically significant. To that extent it supports the view that the venation of the blade has been developed, probably both in phylogeny and ontogeny, somewhat in relation to the problems of extravascular transfer through living foliar tissues.

DISCUSSION.—Evidence has been cited (6, 10) indicating that the venation in leaves of certain dicotyledons is capable of conducting water in amount that greatly exceeds normal requirements. While the transfer of liquids through veins is demonstrably very rapid and according to Mer (1910) with seemingly little resistance, movement of materials through living cells is necessarily slow. There is a marked difference between the mass movement of liquids in tracheal tubes and a slow diffusional transfer by living cells even if accelerated by protoplasmic streaming. Though veins may have a water carrying capacity in excess of current need the service area of a strand is obviously related to the facility of local transfer to or from these channels by associated nonvascular tissues. This operates to limit the effective distance between veins and emphasizes their importance for critical distribution as well as for conduction to or from various regions of the blade; it also shifts attention from the vascular to the nonvascular movement of materials in the blade.

Under such limitations the number, size, shape and arrangement of living cells between veins may influence the rate of intervascular transfer and thereby become a potential factor in vein spacing for that blade. The spongy mesophyll in particular seems favorable for such transfer between vascular strands. Its cells have broad areas of contact and are usually elongated in the plane of the blade while their arrangement in superimposed meshes favors movement in any direction.

The palisade presents a contrasting situation; with slender cylindric cells at right angles to the epidermis this tissue involves conductive problems somewhat proportional to its relative volume. This situation was clearly appreciated long ago by Haberlandt (1914) who even held that there was no lateral transfer between palisade cells. Turrell (1936) has shown that the palisade exposes extensive areas of moist cell wall against intercellular space, yet this tissue, the center of photosynthetic activity and the locus of the leaf's greatest transpiration, lacks favorable vascular connections and must be largely sustained by contiguous tissues. The complementary sponge, when present in sufficient amount doubtless carries much of this responsibility but other blade tissue may help in varying degree.

The epidermis under certain conditions may aid to some extent with these intervascular problems. Wylie (1943) has reported evidence that in certain mesomorphic leaves the epidermis, if given supply by closely spaced vein-extensions, might aid in water movement. A much smaller proportion of these Florida leaves presented similar conditions. Relatively fewer of them have close association of vein-extensions and in some the epidermal cells are partly separated by cuticular wedges. In such leaves the epidermis, across considerable areas of blade, is necessarily supported and supplied by the underlying mesophyll. Compensating factors are the thicker epidermis in many of these leaves, multiple epidermal layers in some, and the frequent presence of compact subdermal cell layers.

The degree of correlation in this series between blade-thickness and vein-spacing (0.3298 $\pm$ .06) may be due to the combined effects of several factors. Important among these is the greater compactness of the palisade, often noted when this tissue is present in considerable amount. Also, in many of the thicker leaves of this series, there is a relatively high proportion of sponge. For the thickest leaf measured, *Calophyllum calaba,* with a blade thickness of 402 μ, the sponge contributed 55 per cent of its volume and the intervascular interval was 231 μ.

About one-tenth of these leaves had subdermal layers, often referred to as water storage tissue. These cells are not only large but are broadly flattened together and are usually without chloroplasts. While such tissue could provide for storage of water in some degree, the amount thus held in reserve

would only briefly cushion transpiration demands. Our inclusion of this tissue with the sponge series seems logical and experiment may reveal its importance for water transfer as well as storage. These zones of subdermal tissue are generally associated with a relatively thin epidermis and a diffuse spongy mesophyll as in many species of *Ficus*.

In evaluating vascularization, the use of vein-spacing is simpler and may have other advantages over that based upon measurement of total vein length per unit area. Both methods involve common problems related to sampling, but they differ in principle as well as in procedure. Measurements of vein length include all veins of given areas, though larger major veins necessarily differ from the lesser in spatial relations, both in the area occupied and in that dominated on either side of these veins. The intervascular interval is readily measured from paradermal sections, which permit also the selection of intervals between veins of equal rank. When possible the spaces measured should be those between veins of the smallest category, or if others must be included, a consistent proportion of types should be used. The smaller minor veins are of special significance because they develop late in blade ontogeny. They seem to represent the final structural adjustments among vascular tissues of the blade; their distribution therefore has special significance in the functioning of that particular leaf.

Further reduction in the organization of minor veins for such leaves is difficult due to the importance of a border-parenchyma about each vein. Armacost (1944) has recently discussed this intermediary sheath which greatly increases the area for mesophyll contacts, and serves, as noted by MacDaniels (1944), "to prevent entrance of air into the vessels, if the pull of transpiration is to be transmitted to the water column in conducting water to any height."

Of significance in this study is the heterogeneous character of the leaves surveyed. The question of sampling, other than the number of species involved, need not be considered because each blade was a survey unit. No attempt was made to establish criteria for any type or species. With more precise evaluation of tissues the study might well have been limited to one species or even to the leaves of a single plant. The only question raised was the degree of correlation between vascular distribution and organization of the nonvascular tissues of the blade. Any seeming overvascularization of foliage leaves is not supported by these findings even though veins actually present could supply much greater areas of blade, because their functions necessarily include elaborate distribution as well as mass conduction to the parts concerned.

The degree of correlation, for the leaves of this series, between tissue organization and vein spacing supports the possibility that problems of transfer through intervening nonvascular tissues are related to vascularization. When variations in the nature and amounts of living tissue between veins, are, in the large, demonstrably associated with the spacing of vascular strands, it suggests that conditions outside the veins themselves may influence their spatial relations in the blade.

SUMMARY

This paper records the results of a tissue survey of leaves from ninety tropical and subtropical species of dicotyledons collected in southern Florida. All blade tissues other than veins were measured and classified into two groups according as the size and arrangement of their cells seemed likely to be favorable or unfavorable with respect to transfer of materials between veins. One group included tissues having their cells closely united and often expanded in the plane of the blade (sponge, epidermal layers, etc.). In the other were tissues with cells elongated vertically (palisade) and any other layers having limited lateral contact between cells.

The relative volumes of these two groups of tissue, for a given leaf, yielded a ratio which was usually related to the vein spacing of that blade. This relation was apparent when all of the species were grouped into classes, separated by given increments in intervascular spacing, as in table 1. Vascularization of these leaves was evaluated in terms of the mean separation of their minor veins; these veinlets, formed later in ontogeny, probably represent the final adjustments between vascular and nonvascular tissues for that leaf. The method for measurement of vein spacing is described. Statistical analysis of full data for all species revealed a significant coefficient of correlation ($0.8855 \pm .02$) between the organization of nonvascular tissues and vein spacing. This supports the view that the distribution of vascular strands in the blade is influenced in some degree by problems of transfer through the living tissues lying between veins.

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PLASTID PIGMENTS AND COMMERCIAL QUALITY IN CONNECTICUT SHADE TOBACCO [1]

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It is difficult to find an industry where scrutiny of the character of a dead leaf is so important commercially as in the evaluation of cigar wrapper. The worth of a wrapper depends on many considerations; color and its distribution over the leaf surface are not the least of these. Definition in measurable terms of the factors which determine quality would be valuable to industry as well as to science and presents fascinating problems in physiology. The Agricultural Marketing Service, U. S. Department of Agriculture, provides the best available definition of the commercial properties of Shade tobacco (1934).

There is apparently no published work on green and yellow pigments of fermented Shade tobacco in relation to leaf quality and the commercial sorting system. However, the literature on leaf plastid coloring matters includes some tobacco work.

Arnaud, who, according to Palmer (1922), was the first to propose a colorimetric method for quantitative estimation of carotene in plant tissues, reported on carotene content of air-dried tobacco leaf in 1889. This datum is listed by Palmer.

Several writers, Elmer (1925), Dunlap (1928), Peterson (1930, 1931), Peterson and McKinney (1938), deal with plastid pigments of fresh leaf in relation to mosaic disease.

Strain (1938, p. 91) reported that normal green leaves of tobacco, among other plants, were found to contain the same xanthophylls in approximately the same proportions as found in barley leaves.

Vickery, Pucher *et al.* (1931, 1933) measured decreases of the amount of ether-soluble material in the curing of Connecticut shade-grown tobacco.

MacKinney (1938) killed tobacco leaf by different methods and observed the effect upon absorption spectra of leaf extracts.

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McHargue, Woodmansee and Rapp (1942) measured the chlorophyll content of low-grade, dark fire-cured tobacco.

Nagel (1939) describes pigment processes, chiefly in fresh tobacco leaves from pot experiments. He found no trace of chloroplast coloring matter in brown leaves which died on the plant. (None of the leaves in the studies here reported died on the plant.)

Smirnov (1933) presents a comprehensive review of the biochemistry of tobacco curing and fermentation.

Street (1930) studied curing temperature and humidity favoring optimum coloration in Connecticut shade-grown tobacco.

Barta and Marschek (1938) show reduction in chlorophyll content of tobacco during fermentation.

Wenusch (1941) stresses the role of chlorophyll decomposition products in coloration of commercial tobacco. Sreerangachar (1943), concerned with the related problems of tea production, explains the brown color of infused tea leaf as due to chlorophyll decomposition products and colored tannin oxidation products.

In the tobacco industry of the Connecticut Valley, "black" tobacco is the term applied to a dull-surfaced, low quality leaf that cures very dark brown with a blue-gray or purple-gray hue. Black tobacco is worthless for fine cigar manufacture and at times has been a considerable loss to the grower, frequently where new land, recently cleared or for many years fallow, has been cropped to tobacco.

Black tobacco should not be confused with the natural dark grades which come from the upper, immature part of the plant. The black tobacco discussed here very commonly comes from the lowest six leaves picked which normally cure to the lightest colors. Packers of shade tobacco usually put black leaves in a separate grade often designated as "blue-black."

Previous work on "black" Connecticut shade-grown tobacco by LeCompte (1941, 1943, 1944) revealed striking contrasts in the color of fer-

TABLE 1. *Color and metal content of light and "black" fermented Shade tobacco from different farms and seasons, in relation to pigments extracted by petroleum ether and methanol from 10 g. dry, powdered leaf web.*

Sample number	Commercial grade or type of tobacco	Leaf color Ridgway code	Per cent dry weight		Petroleum ether[a] Dilution	Petroleum ether[a] Transmission, per cent at 425 mμ		Ethyl ether[b] Dilution	Ethyl ether[b] Transmission, per cent at 425 mμ	
			Fe	Mn	ml.	Fisher[c]	Beckman[d]	ml.	Fisher[c]	Grower[e]
36	Black	XXIX 17″m	.12	.17	100	84.6	96.0–96.5	50	84.0	A
44	LC2, Light	XXIX 17″k	.09	.04	100	49.3	54.6–57.2	100	76.5	
15	YL, Light	XV 17′k	.05	.03	100	36.0	.....	50	18.9	C
16	"Black"	XXIX 17″m	.07	.10	100	81.0	.....	50	80.4	

[a] Yellow pigments remaining after saponification and washing with 90 per cent aqueous CH_3OH.
[b] Yellow pigments from saponified aqueous methanol transferred to ethyl ether.
[c] Fisher electrophotometer, cylindrical absorption cells, *ca.* 2.27 cm. diam.
[d] Beckman spectrophotometer, pyrex test tubes *ca.* 1 cm diam.
[e] Reference to table 28, Conn. Agric. Expt. Sta. Bull. 444. 1941.

mented leaf as related to tissue content of iron and manganese and to soil environment.

Black tobacco was traced to fields which were excessively acid and deficient in "available" phosphorous and calcium. Per unit weight of dry matter, black leaf generally contained notably more manganese and iron than light, good quality leaf. It was assumed that excessive absorption of Mn and Fe and development of the black pigment bore some inter-relationship, though not necessarily one of cause and effect. Field experiments showed that black tobacco was eliminated and satisfactory quality of light-leaf was obtained after heavy phosphorus fertilization over a two or three year period. Heavy liming by itself merely lightened the color of the cured leaves and did not produce certain other good qualities.

In order to throw light on the relationship between commercial quality of Shade tobacco and coloring matters extractable from black and light leaves, the following experiments were made in the winter of 1943–44 at the Connecticut Agricultural Experiment Station, Windsor, and New Haven, Connecticut.

Usually the percentage transmissions of pigment solutions and of pure solvent were compared in a Fisher Electrophotometer. Cylindrical absorption cells were stoppered with clean corks. Transmissions determined with a Beckman spectrophotometer are so designated in the tables. Solvents and other chemicals of reagent grade were used without further purification.

The color of the powdered tobacco sample was matched under daylight from a north sky as closely as possible with Ridgway's color standards (1912), and is identified by Ridgway's code. In a few cases, plus signs have been added to indicate a shade slightly darker than the Ridgway color. If desirable the Ridgway colors may be defined according to the Munsell system (1939) as described by Judd and Kelly (1939), and Kelly (1940).

Iron and manganese contents of the tobacco are

TABLE 2. *Color, Fe and Mn content of fermented Shade tobacco in relation to average transmission of yellow pigments[a] in solution[b],*

Sample number	Commercial grade or type of leaf	Leaf color Ridgway code	Transmission (per cent) of solution at 425 mμ Petroleum ether	Transmission (per cent) of solution at 425 mμ CH_3OH 90%	Metal content Per cent dry weight Fe	Metal content Per cent dry weight Mn	Reference[d]
1	YL, Light	XV 17′k	77.3	64.3	.05	.04	Table 28
6	YL, Light	XXIX 17″k	79.1	71.3	.03	.01	Grower A
2	KV, Black	XL 21‴k	91.1	87.0	.20	.19	
3	"Black"	XL 21‴k	91.5	86.8	.13	.19	
117	V1	XXIX 17″k	65.4[c]	...	.10	.05	
118	V2	XXIX 17″k+	73.2[c]	...	.12	.06	Table 31
119	KV1	XXIX 17″k++	74.1[c]	...	.13	.10	
120	KV2	XL 17‴k	73.9[c]	...	.17	.14	
121	"Blue Black"	XXX 19″m	83.4[c]	...	.20	.16	

[a] One gram dry, powdered leaf extracted by method for carotene, Official and Tentative Methods of Analysis, A. O. A. C., 5th Edition.
[b] Final volume of solutions, 100 ml. for both petroleum ether and 90 per cent CH_3OH.
[c] Duplicate tests, total yellow pigments soluble in petroleum ether.
[d] Reference to tables 28–31, Conn. Agric. Expt. Sta. Bull. 444. 1941

presented where possible for comparison with pigment data and, if previously reported, are so marked.

YELLOW PIGMENTS.—For a preliminary survey of extractable yellow pigments, 10-gram powdered samples of tobacco of contrasting color from several growers were shaken at intervals for 20 days in a darkened room with a mixture of 100 ml. petroleum ether (b.p. 30° to 60°C.) and 50 ml. absolute methanol. Extracts were filtered through nonabsorbent cotton and transferred to separatory funnels. Upon separation the methanol fractions were run off into other funnels and washed with 20 ml. petroleum ether. Similarly, the first petroleum ether fraction was washed with 90 per cent aqueous methanol. Petroleum ether fractions were combined, shaken with 5 ml. methyl alcoholic KOH and, after discarding the saponified layer, extracted again with 90 per cent methanol. The petroleum ether fractions were washed with water, dried over Na_2SO_4 and made to volume. All aqueous methanol washes were combined with the absolute methanol extract, shaken with 5 ml. methyl alcoholic KOH, diluted with water and extracted with ethyl ether. The ether solutions were washed with water, dried over Na_2SO_4 and made to volume. Data are presented in table 1 where commercial grade, color, and metal content of the leaf tissue are compared with concentration of extracted pigment in solution.

Where dilutions are alike the contrast of the yellow pigments from black tobaccos as opposed to those from light tobaccos is striking. Much more yellow pigment (low transmission values) was derived from light tobaccos than from black tobaccos (high transmission).

Table 2 presents examples of data from a systematic comparison of light and black tobaccos from different fields. Triplicate, one-gram powdered samples of tobacco were extracted by the tentative method for carotene (Association of Official and Agricultural Chemists, Methods of Analysis, 5th Edition, 1940). The 90 per cent methanol washes of samples 1, 6, 2, 3 were not discarded, as in the tentative method, but were combined, filtered, and made to volume, constituting a "xanthophyll" fraction. "Xanthophylls" were not separated from "carotins" of samples 117 to 121, inclusive.

Average transmissions are presented. Deviations from the mean were usually less than 3.5 per cent of the mean value. However for one deep yellow solution (table 3, 57.4 average transmission in 90 per cent methanol) there was 10.6 per cent deviation.

Table 2 shows that dark tobacco yielded less yellow pigment than light tobacco from any one field. The progressively darker grades V1, V2, KV1, KV2, "blue black," represented by samples 117 to 121, yielded almost progressively less yellow pigment.

Table 3 compares the extreme of light (LL) and dark (KV2, KVB) grades with a mid-toned grade (V) from two different plots in respect to yield of "carotene" and "xanthophyll" by a modification of the method of The Association of Official and Agricultural Chemists (1940). The modification consisted in using alcoholic NaOH instead of alcoholic KOH. Single transmission measurements are denoted. Otherwise, transmission data are averages of triplicates. More yellow pigment was generally obtained, the lighter the grade or color of tobacco.

Table 4 shows the effect of successive A. O. A. C. (with NaOH) extractions on single, one-gram samples. Very little yellow pigment appears after the second extraction. Table 4 also demonstrates quite regularly a smaller yield of yellow pigment, the darker and less valuable the commercial grade. Transmission data represent total "carotinoids" in petroleum-ether, the "xanthophylls" not having been separated.

A greater amount of total "carotinoid" was extractable from green, quick-dried Shade tobacco in contrast to the smaller amount of total carotinoids from fermented tobaccos.

Deterioration of the pigments in petroleum ether stored at 20° to 26°C. in the dark was indicated by a rise in transmission values after 31, 42, and 53 days. After 31 days storage, however, solutions from fermented tobaccos showed little or no deterioration.

Ethyl alcoholic KOH and NaOH at two concentrations were compared in their extractive effect on total carotinoids from identical grades of fermented tobacco. Irrespective of which alkali or concentration was tested, extremes of the commercial grade series (light LC or LC2 as opposed to black KV2) showed considerable contrast in the amount of total carotinoids, the darker the grade the less carotinoid pigment being generally extractable from it.

Only freshly prepared, colorless alcoholic alkali was used in extraction. Stale alcoholic alkali with a brown color was avoided because it could give deceptive transmission blank-values in petroleum ether which might erroneously be ascribed to carotinoids. The brown color of stale alcoholic alkali may be due to interaction of alkali and aldehydes with formation of resinous polymers that are soluble in petroleum ether.

Less "carotinoid" pigment was extracted from dark brown areas than from light brown areas of single leaves.

GREEN PIGMENTS.—For the purpose of comparing light (LL, YL1, LC, LC2) and dark (V2, KV, KV2) fermented Shade tobacco from several sources, table 5 presents average transmission in the red (650 mμ) and blue (425 mμ) regions of the spectrum of extracts in 85 per cent aqueous acetone. Extracts of 1 gram dry tobacco were prepared in triplicate by the tentative method for chlorophyll (Association of Official Agricultural Chemists, 1944). Extraction in a Waring Blendor for 5 minutes, filtering through paper on a Büchner funnel, and repeated washing of the residue on the funnel, permitted dilution of the filtrate to 250 ml.

TABLE 3. *Commercial quality and color of fermented Shade tobacco in relation to average transmission of yellow pigments*[a] *in solution*[b].

Sample number	Commercial grade of leaf	Leaf color Ridgway code	Transmission, per cent of solution at 425 mμ		Reference or source of leaf
			Petroleum ether	CH_3OH 90%	
171	LL	IV 19 m	69.6	57.4	Farm A[c]
163	V[d]	XXIX 17″k	78.2	59.7	Leaves 4–6
160	KVB	XXX 19″k	83.3	72.9	1941 crop
182	LL	XXIX 15″k	73.9	62.2	Farm B[c]
175	V[d]	XXIX 17″k	78.4	69.0	Leaves 4–6
172	KV2	XXIX 17″k	89.7	83.5	1941 crop

[a] One gram dry, powdered leaf extracted by alcoholic NaOH, otherwise method for carotene, Official and Tentative Methods of Analysis, A. O. A. C., 5th Edition.
[b] Final volume of solutions, 100 ml. for both petroleum ether and 90 per cent CH_3OH.
[c] Reference to test plots of LeCompte (1943).
[d] One test.

Final washings of the residue with 85 per cent acetone were nearly colorless. Some extracts showed a red fluorescence. Photometric transmissions were determined immediately after preparation of the extract.

Table 5 shows that chlorophyll components gave transmission values in the red (650 mμ) which are lower for dark tobaccos than for light tobaccos in five out of eight comparisons on fermented leaf. This means that about 60 per cent of the dark samples examined yielded more green pigments than comparable light samples. Deviation from the mean of the transmission values (650 mμ) of table 5 were about 1 per cent to 2 per cent of the average value. At 425 mμ, deviations from the average transmission were generally about 2 to 3 per cent and did not exceed 6.1 per cent of the mean.

On the other hand, transmission in the blue for seven out of eight comparisons showed a lower value for light tobacco than for the companion (check) black tobacco. This is evidence which tends to corroborate carotinoid measurements by other extraction methods of tables 1 to 4. However, 85 per cent aqueous acetone extracts of dry plant tissues might contain water-soluble flavones and therefore would not necessarily provide reliable information on carotinoid content of a leaf sample.

DISCUSSION.—The yellow and green pigments from fermented Shade tobacco have not been identified chemically. They are defined in this paper only by the per cent transmission of light through their solutions. Their behavior toward various solvents indicates that they are probably plastid pigments or decomposition products thereof. However, to report the substance which remains in petroleum ether after following the tentative method for carotene (Association of Official Agricultural Chemists, 1940) as *carotene* without confirmatory chromatographic, spectrometric or chemical evidence would be obviously unsound. The yellow pigments extracted from leaves of contrasting color and commercial quality, tables 1 to 5, may differ widely in chemical structure. It would be remarkable if, for example, the yellow pigments extracted from a black leaf on the one hand and from a light leaf on the other should differ in concentration only and be chemically identical.

It is unknown to what extent the carotinoids

TABLE 4. *Transmission of yellow pigments*[a] *in petroleum ether*[b] *from successive extraction of fermented Shade tobacco as related to commercial grade and leaf color.*

Sample number	Commercial grade[c]	Leaf color Ridgway code	Transmission, per cent at 425 mμ after successive extraction			
			1st	2nd	3rd	4th
171	LL, Light	XV 17′k	37.0	84.0	93.5	...
170	LC	XV 17′k	43.4	82.9	93.4	...
168	YL	XV 17′k	47.9	79.7	93.0	...
166	LV	XXIX 17″k	49.2	86.7	82.8	92.2
164	LV2	XXIX 17″k	48.4	77.5	86.2	90.3
163	V	XXIX 17″k	53.9	69.8	89.0	89.0
162	KV	XXIX 17″k	54.2	...	...	...
161	KV2	XXIX 17″k	56.4	...	...	...
160	KVB, Black	XXX 19″k	70.9	...	...	...

[a] Total yellow pigments, soluble in petroleum ether, from successive extractions with alcoholic NaOH of 1 gram dry, powdered leaf, plot 13, leaves 4–6, Field A, 1941 crop cf, LeCompte (1943) p. 150.
[b] Final dilution, 100 ml., all single tests.
[c] Money value decreases down the column.

TABLE 5. *Average transmission (per cent) of extracts of 1 g. dry Shade tobacco in 85 per cent aqueous acetone as related to commercial grade, tissue color and content of iron and manganese.*

Sample number	Tobacco, commercial grade or type	Transmission, per cent at 650 mμ	Transmission, per cent at 425 mμ	Dilution of acetone extract, ml.	Per cent dry weight Fe	Per cent dry weight Mn	Leaf color Ridgway code
6	YL1, Light	48.4	18.1	250	.03	.02	XXIX 17″k
2	KV, Black	60.4	37.8	250	.20	.19	XL 21‴k
20	"Light"	56.0	24.3	250	.03	.04	XV 17′k
21	"Black"	49.1	27.0	250	.06	.09	XXIX 17″m
64	"Light"	71.9	40.9	250	.15	.03	XV 17′k
66	"Black"	73.6	45.3	250	.29	.12	XXIX 17″m
76	"Light"	73.6	41.0	250	.30	.03	XV 17′k
78	"Black"	61.2	39.6	250	.35	.10	XL 17‴k
170	LC, Light	49.3	17.5	250	..	..	IV 19m
161	KV2, Black	46.9	20.0	250	..	..	XXIX 17″m
182	LL, Light	65.4	28.8	250	..	..	XV 17′k
173	KV, Black	58.0	31.6	250	..	..	XXIX 17″k
131	LL, Light	55.9	15.8	250	..	..	IV 19m
123	V2, Dark	56.3	21.3	250	..	..	XL 17‴k
169	LC2, Light	45.6	13.9	200	..	..	IV 19m
161	KV2, Black	44.5	16.2	200	..	..	XXIX 17″m

contribute to the commercial quality of tobacco. The experiments described in this paper suggest that they may play some part. Wenusch (1941) states that a yellow tobacco lost its luster or sheen after exhaustive extraction with chloroform and alcohol, although, except for this effect, the color of the sample was not greatly changed. Wenusch concludes that neither carotene nor xanthophyll nor both together are the main source of the yellow coloration. Further work is needed involving accurate color measurements, *e.g.,* by spectrometric reflectance, to show what effect each extractable pigment has on the general appearance of a tobacco.

Comparison of the colors by instrumental methods free of visual errors would doubtless make fine distinctions possible. Nevertheless, visual estimates of powdered samples have been attempted, in the lack of a more precise method, and have at least permitted rough definitions of tobacco color. The Ridgway data are presented only as approximate color matches. For the practical tobacco man the best criterion of quality is the commercial grade name such as L or KVB. But these terms are not absolute or objective, as tobacco experts realize. This has been discussed by LeCompte (1943).

On the basis of equal dry weights, light colored tobaccos of tables 1 to 5 usually yielded more yellow pigments than comparable black tobaccos (from comparable plots, farms and seasons) but at the same time contained less of the heavy metals Fe and Mn than the black samples. This fact raises the question whether the basis of dry weight is valid for comparisons of plastid pigment contents. Since plastid pigmentation is closely related to leaf area or tissue volume it is logical that similarly pigmented tobacco with incomparable specific gravities would yield different quantities of pigments if equal weights of dry tissue were tested. The observations of Peterson and McKinney (1938) on mosaic-infected tobacco may apply here. These workers found "green weight" of tobacco leaves comparable to area as a basis for pigment measurements. Dry weight was less comparable to area because of *the relatively low dry weight of yellow mosaic plants.* It is a fact that superior Shade tobacco has thinner and more tissuey body than inferior tobacco. Body is defined as weight per unit of surface by the U. S. Department of Agriculture (1934). Whether specific gravity and body of tissue vary directly is undetermined.

The pigment data in table 5 show in several cases comparable yield of extractable green matter from both light and black tobacco. Sometimes even more green pigment was extracted from the black sample than from the light sample. We cannot assume, therefore, that differences in specific gravity of dry tobacco tissue might be neglected for comparisons of plastid pigment contents. It is not known whether the green and yellow coloring matters are extractable at comparable rates and in proportion to their concentration in the leaf. Differences have been measured in the amount of pigment extractable from light and dark sections of identical leaves. Here, perhaps, differences in specific gravity may be neglected.

The data of tables 1 to 5 appear to present a dilemma: either the differences in yield of yellow pigment between grades are valid on a dry weight basis or a minute distinction between the specific gravities of commercial grades has been demonstrated and the differences in yellow pigment yield are only apparent.

Chemical data reported here and elsewhere (LeCompte, 1941, 1943, 1944), apply to *commercial*

tobacco and not solely to leaf tissue, free of foreign matter. Contamination with soil particles and other material is involved but is believed to be approximately constant, irrespective of commercial grade, for leaves picked at the same level above the soil.

SUMMARY

Light, black and intermediate grades of Connecticut shade-grown fermented tobacco have been compared, on the basis of equal dry weights of plant tissue, for extractable yellow and green pigments.

Yellow pigments were extracted by three methods, namely, (1) shaking at room temperature with a mixture of petroleum ether and methanol, (2) refluxing with ethyl alcoholic KOH or NaOH and (3) comminution with 85 per cent aqueous acetone in a Waring Blendor. Generally, the better the commercial grade or the lighter the color of the leaf, the greater was the amount of yellow pigments extractable and *vice versa*. This relationship was found even between differently colored areas of similar or identical leaves.

More green coloring matter was extracted by 85 per cent acetone from dark tobacco than from light tobacco in five out of eight comparisons.

Analyses reported here and elsewhere (LeCompte, 1941, 1943, 1944), apply to commercial tobacco and not merely to the leaf tissue itself. Contamination with foreign matter is involved but is believed to be approximately constant, irrespective of commercial grade, for leaves picked at the same level above the soil.

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A MORPHOLOGICAL, DEVELOPMENTAL, AND CYTOLOGICAL STUDY OF FOUR SAPROPHYTIC CHYTRIDS. IV. PHLYCTORHIZA ENDOGENA GEN. NOV., SP. NOV. [1]

Anne Marie Hanson

THIS MONOCENTRIC rhizidiaceous inoperculate chytrid occurs in the integuments of various insects in nature. It is characterized by an intramatrical flattened sporangium with a basal discharge papilla, and a subsporangial radiate system of anastomosing rhizoids which may frequently become locally vesiculate. The general appearance of the mature thallus with its radiate rhizoids is superficially similar to that of *Rhizophlyctis,* but inasmuch as the sporangium appears to originate intramatrically by vesiculation of the branched germ tube or incipient rhizoids, it cannot be included in this genus as *Rhizophlyctis* is interpreted at present. Likewise, it does not appear to belong in any of the known chytrid genera, and for this reason it is diagnosed as a new genus and species for which the name *Phlyctorhiza endogena* is proposed.

Phlyctorhiza n. gen.—Thallo monocentrico, eucarpico, intramatricali. Zoosporangiis augentibus ut continuatio tubulae germinis, zoospore generatim manente ut tuber. Systemate rhizoidiali super sporangii basi et peripheria constituto, frequenter conjuncto per anastomosin. Zoosporangiis variatim formatis habentibus unam papillam exeuntem, vel a basi, vel a latere, vel ab apice. Zoosporis a posteriore uniflagellatis. Sporis perdurantibus variatim formatis, pariete crasso praeditis et evidenter augentibus eodem modo ac sporangia; germinatione complete, ut prosporangia agunt.

Phlyctorhiza n. gen.—Thallus monocentric, eucarpic, intramatrical. Zoosporangia developing as an outgrowth of the germ tube while the zoospore usually persists as a cyst. Rhizoidal system oriented on the base and periphery of the sporangium, frequently anastomosing. Zoosporangia variously shaped, with one basal, lateral, or apical exit papilla. Zoospores posteriorly uniflagellate. Resting spores variously shaped, thick walled, and apparently developing in the same manner as the sporangia; upon germination functioning as prosporangia.

P. endogena, n. sp.—Zoosporangiis hyalinis, levibus, libratis, depressis, generatim figura ad modum phaseoli, 6 × 10–33.6 × 58.2 μ, saepe ovatis, 7 × 12–26.2 × 42 μ, irregularibus, 11 × 20–29 × 36 μ; habentibus unam papillam exeuntem a basi, raro a latere vel ab apice, 2.9–4 × 5.2–7.5 μ diametro. Zoosporangiis augentibus ut continuatio tubulae germinis, zoospore generatim manente ut tuber. Systemate rhizoidiali super sporangii basi et peripheria constituto, saepe vesiculato ad punctum originis, frequenter conjuncto per anastomosin, extendente a 15 ad 300 μ ultra sporangium; partibus rhizoidiis vesiculatis 3–8 μ diametro. Zoosporis ellipticalibus, 2.2–2.9 × 2.9–3.7 μ, uno globulo refractivo centrico vel excentrico; flagello 20–26 μ longitudine emergentibus et enantantibus in vesicula tenui. Sporis perdurantibus ovatis, 8.6–12 × 15–20 μ, reniformis 8.2–16.4 × 10.4–21.6 μ, irregularibus, 12–21.2 × 24–29 μ, praeditis parietibus colore luteo ad sucineum, levibus undulatis vel tuberculatis, 0.746–1.5 μ crassitudine, rebus contentis dense et crasse granulatis; germinatione complete, ut prosporangia agunt.

P. endogena, n. sp.—Zoosporangia hyaline, smooth, flattened, depressed, usually somewhat kidney-shaped, 6 × 10–33.6 × 58.2 μ, frequently oval, 7 × 12–26.2 × 42 μ, irregular, 11 × 20–29 × 36 μ; with one basal, rarely lateral or apical exit pore, 2.9–4 × 5.2–7.5 μ in diameter. Sporangia developing as an outgrowth of the germ tube while the zoospore usually persists as a cyst. Rhizoidal system oriented on the base and periphery of the sporangium, often vesiculate at point of origin, frequently anastomosing, extending from 15–300 μ beyond the sporangium; vesiculate portions of rhizoids 3–8 μ in diameter. Zoospores elliptical, 2.2–2.9 × 2.9–3.7 μ, with one centric or excentric refractive globule; flagellum 20–26 μ long, emerging and swarming in a vesicle outside of the sporangium. Resting spores oval, 8.6–12 × 15–20 μ, reniform, 8.2–16.4 × 10.4–21.6 μ, irregular, 12–21.2 × 24–29 μ, with light golden to deep amber, smooth undulate, or tuberculate walls, 0.746–1.5 μ thick; contents densely and coarsely granular; upon germination functioning as prosporangium.

This chytrid was first observed in mosquito integuments from a small stream near Lakewood, N. J., and later collected in remains of mayflies, dragonflies, and gnats in a brook near Sharon, Conn. From the latter source it was cultured successfully for more than two years in the laboratory on cockroach integuments suspended in filtered brook water. Attempts were made to culture it on strips of purified shrimp chitin as well as 0.5 per cent shrimp, lobster, and 17-year locust chitin-agar without success. Likewise, it failed to grow on Leitner's, Saboraud's, potato dextrose, corn meal, glycogen, and plain agar. Thus far, it has been found to grow only in the integuments of various insects. Although it accordingly appears to be chitinophilic in its nutrient requirements, this is by no means certain. As shown in fig. 2 and 38, the chytrid does not grow in the chitinous layers of the integument but in the basement membrane, which according to Wigglesworth (1939), Snodgrass

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The author expresses her gratitude to Reverend Edwin A. Quain, S.J., Ph.D., of Fordham University for the Latin diagnosis.

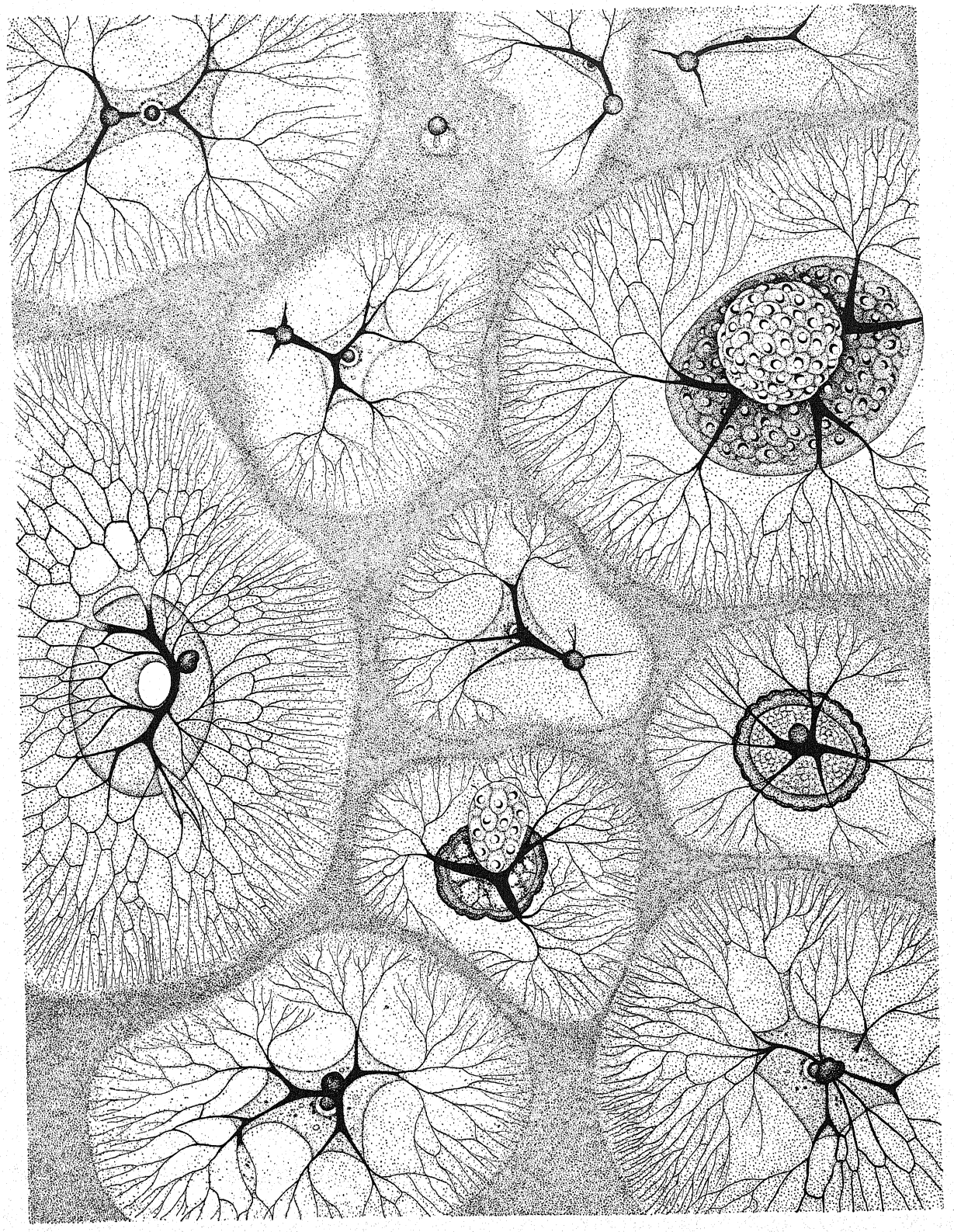


Fig. 1. Surface view of a portion of the basement membrane with thalli of the fungus in various stages of development. ×1580.

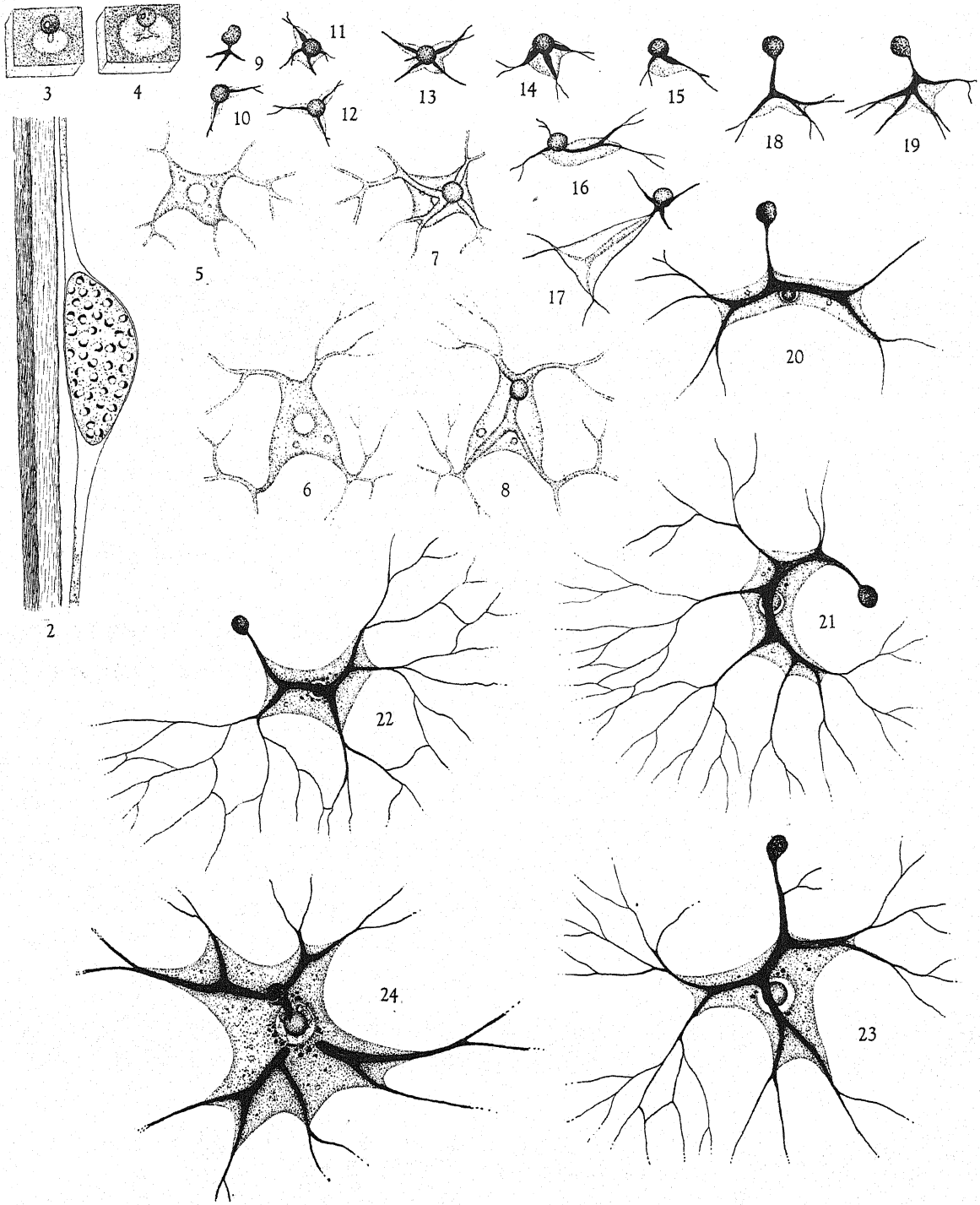


Fig. 2–24.—Fig. 2. Cross section through the cockroach integument showing a sporangium of the fungus distending the layers of the basement membrane. ×1180.—Fig. 3, 4. Germination of zoospore and development of rudimentary rhizoids. ×1580.—Fig. 5, 6. Dorsal views of two fairly advanced thalli. ×1580.—Fig. 7. Ventral view of thallus shown in fig. 5. ×1580.—Fig. 8. Ventral view of thallus shown in fig. 6. ×1580.—Fig. 9–24. Early developmental stages of thalli showing the relationship between the zoospore cyst, rhizoids, and incipient sporangium. All are ventral surface views with the exception of fig. 17, which is a dorsal surface view. ×1580.

(1935), and Imms (1934) may possibly be composed of connective tissue. In the cockroach, this membrane measures 1.8–2 μ in thickness, and as the chytrid thallus develops and spreads, the membrane is expanded (fig. 2, 38) and absorbed so that in surface view each thallus appears to lie in a clear

area (fig. 1). However, the outer and inner surfaces of the basement membrane remain intact and cover the thallus above and below.

Optimum microscopic preparations are obtained by the use of Nawaschin's fixative and Flemming's triple stain when used *in toto*.

DEVELOPMENT OF THE THALLUS.—In germinating, the zoospore forms a stout germ tube which penetrates the surface of the basement membrane (fig. 3) and soon develops several short, irregular branches (fig. 4). The immediate subsequent developmental stages are difficult to observe in living material because of the minuteness of the thalli and relative opacity of the integument. Larger thalli, however, show that the thalli are dorsoventrally differentiated. The dorsal surface is *Rhizophlyctis*-like (fig. 5, 6), but the ventral surfaces of the same thalli are not inasmuch as these are traversed by rhizoids.

The origin of the sporangial rudiment is not definitely known, but incipient thalli fixed and stained *in toto* indicate that it begins as a unilateral vesiculation of the incipient rhizoids—that is, the dorsal portion of the elongate branches vesiculates while the ventral portion remains unexpanded. The result is the formation of thalli in which the sporangial rudiment has the appearance of a thin flattened extension between the main rhizoidal branches (fig. 10–24). Superficially, the thalli are strikingly similar in appearance to a bat's wing or a duck's foot, with the incipient sporangium corresponding to the web or membrane between the digits, and the ventral portions of the rhizoids corresponding to the digits. This development is difficult to observe and interpret in living material, but if the integuments are fixed and stained *in toto*, the incipient chytrid thalli stand out sharply. In such preparations the persistent zoospore case, germ tube, and the ventral portion of the incipient rhizoids appear deeply stained, while the sporangial rudiment is only faintly so (fig. 9–24).

As the rhizoids grow and spread more or less radially in one plane within the very thin basement membrane, the layers of the latter are spread apart, and vesiculation of the dorsal portion of the rhizoids continues to some extent along the paths opened up by the rhizoids in the substratum. The incipient developing sporangia, therefore, become irregular in shape and in many instances may appear somewhat stellate in surface view (fig. 22–24). However, after vesiculation has ceased, they expand between the distal points and become more regular in shape and almost circular in outline as shown in fig. 25, 32, 33, and 34.

The incipient sporangia usually remain uninucleate until fairly large. The nucleus is not sharply visible in living material, but its position is evident as an almost spherical, relatively hyaline area as shown in fig. 5 and 6. In thalli which have been stained *in toto* it stands out as a densely stained body with a large nucleole (fig. 20–24), and as the incipient sporangium enlarges the nucleus increases correspondingly in size and becomes quite conspicuous (fig. 23, 24). Nuclear multiplication soon takes place (fig. 26–28), so that by the time the sporangia have reached definitive size they are multinucleate.

Frequently, as the sporangium approaches maturity, additional vesiculations of the rhizoids occur, and these appear to develop on the dorsal side of the rhizoids in the same manner as the sporangial rudiment (fig. 30–32). Some of these vesiculations may be continuous with the sporangium (fig. 32, 34), but in other cases they appear to be disconnected (fig. 31, 33). In a few cases the continuous vesiculations were found to contain nuclei (fig. 35, 36). Apparently, during development of such thalli, nuclei from the incipient sporangium migrate into the vesiculations. If they remain there and multiply, and the vesiculations become delimited by septa, secondary sporangia may develop so that the thallus becomes polycentric. Fig. 36 shows such a thallus with one empty primary and thirteen secondary sporangia in various stages of development. However, polycentric thalli are rare and exceptional, and so far only three have been observed. *Phlyctorhiza endogena*, therefore, is predominantly monocentric but occasionally shows a tendency to polycentricity.

As the sporangia mature, their protoplasmic contents undergo the characteristic changes which have been described for most rhizidiaceous chytrids. At least, no marked and unusual differences have been observed. Accordingly, a detailed description and illustration of the changes is not essential.

For spore discharge a broad short papilla usually develops more or less centrally in the ventral wall of the sporangium, but the papilla may occasionally develop in the dorsal or lateral surface of the sporangium. Initially, it is not prominent, but just prior to discharge its outline becomes clearly defined (fig. 37). Cross sections of infected integuments show that the papilla may often penetrate the basement membrane (fig. 38). This is not always true, however, for a great number of sporangia discharge their spores within the basement membrane. With the onset of discharge the spores emerge as a cluster (fig. 39). The cluster rapidly enlarges as more and more spores are extruded but remains firmly attached to the sporangium. Two or three minutes after the cluster ceases enlarging the individual spores begin to glide upon one another, and in about 20 seconds they are swarming rapidly. At this time it is evident that the spores are enveloped by a thin hyaline membrane, for although they swarm violently, they do so in a restricted area and do not disperse. Due to the violent swarming, the vesicle changes in shape continuously as has been described by Karling for species of *Rhizidium* (1944), *Chytriomyces* (1945a), *Rhopalophlyctis* (1945a), and *Siphonaria* (1945b). It may change rapidly from a globular to an elongate sac and then

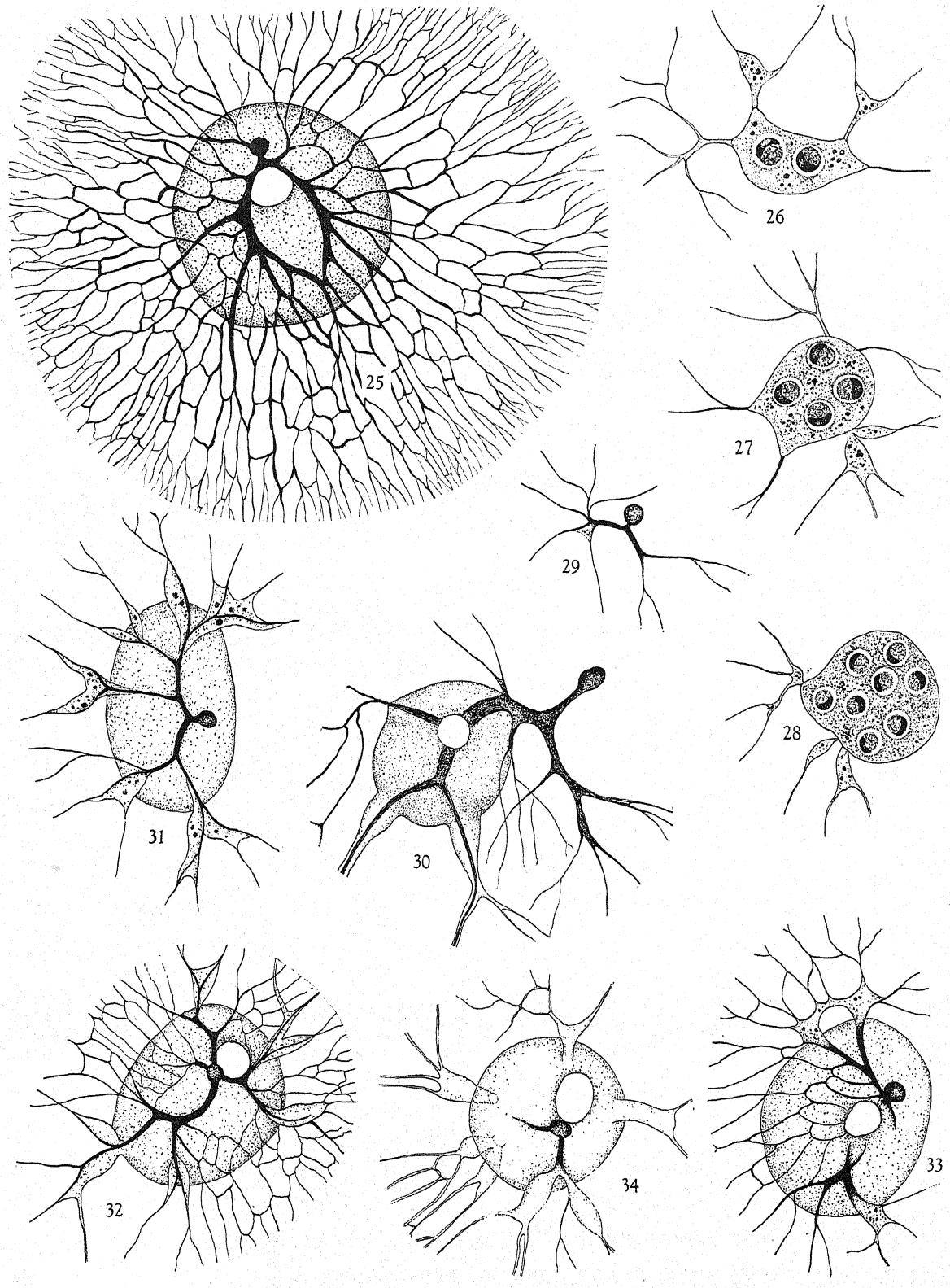


Fig. 25–34.—Fig. 25. Empty sporangium with extensively anastomosing rhizoids. ×1180.—Fig. 26–28. Bi-, tetra-, and eight-nucleate sporangia. ×1580.—Fig. 29. Vesiculation developing distally from rhizoids in the region of branches. ×1580.—Fig. 30. Empty sporangium, which developed from a vesiculation as shown in fig. 27; additional vesiculations of rhizoids continuous with the sporangium. ×1580.—Fig. 31. Sporangium with additional rhizoidal

again revert to globular shape. During these changes in shape, however, the vesicle remains firmly attached to the periphery of the exit orifice. After 5 to 6 minutes of collective swarming, the vesicle is ruptured in local areas and the zoospores begin to escape. They are broadly elliptical in shape (2.2–2.9 × 2.9–3.7 μ) and contain one centric (fig. 40) or lateral (fig. 41) refractive globule. Not all spores escape as a globular mass. Some remain in the sporangium where they later become motile and then escape one by one subsequent to the rupture of the vesicle.

Intra vitam staining of the vesicle in living material with ruthenium red, Janus green, neutral red, and chloroiodide of zinc has not been successful. However, in preparations fixed at the first sign of discharge and stained *in toto* with Flemming's triple stain, a delicate film of material may be seen surrounding the spore mass. This film is believed to represent the vesicle.

In empty sporangia fixed and stained *in toto* the exit papilla appears as a circular (fig. 42–44, 25, 30, 32) or oval (fig. 33, 34) opening, but its relation to the rhizoidal axis is variable. In some instances it develops to one side of the axis but does not appear to affect the latter's structure (fig. 25, 32). In other instances the exit pore may develop right in the region of the main axis of the rhizoidal system. In such cases the overlying part of the rhizoid may be torn away by the emerging spore cluster (fig. 42), but it may also remain intact as a shriveled bar extending over the exit pore (fig. 43). In still other instances the rhizoids terminate smoothly and flush with the periphery of the exit papilla (fig. 44, 30). This suggests that the rhizoidial axis in this region may be dissolved or transformed prior to or during formation of the exit papilla. Such a process is further suggested in young developing thalli in which a part of the rhizoidal axis is lacking in a region where the exit papilla typically develops (fig. 24).

Development and germination of resting spores.—The resting spores of *P. endogena* appear to be formed in a manner similar to the sporangia, and in their early developmental stages cannot be distinguished from the latter. No evidence of sexuality has been observed so far. The resting spores are produced abundantly in old cultures. They appear therefore to be formed under unfavorable conditions, and as is typical of such structures, their walls are relatively thick (0.746–1.5 μ). Generally the walls are tuberculate (fig. 46–49), but they may also be smooth (fig. 45) or undulate. Smooth walls are light golden in color, but the tuberculate walls are deep amber. In shape the resting spores may be oval (8.6–12 × 15 μ), reniform (8.2–16.4 × 10.4–21.6 μ), or irregular (12–21.2 × 24–29 μ). Like the zoosporangia, they are flattened and compressed. Their rhizoids are less extensive than those of the zoosporangia, but as in the case of the latter, they also may be vesiculate (fig. 46). Anastomoses, on the other hand, are infrequent and limited. The content of the resting spore is densely and coarsely granular, but a central hyaline area is sometimes present (fig. 46).

With the onset of germination, a small hyaline papilla emerges basally in the region usually occupied by the exit papilla of the zoosporangia (fig. 47). Gradually, as the contents of the resting spore emerge, the hyaline papilla expands to form an oval or pyriform sporangium (fig. 48). The resting spore, therefore, functions as a prosporangium in germination.

The zoosporangium produced by the germinating resting spore is always of smaller diameter than the spore from which it develops. While its contents mature and zoospores are delimited, a small discharge papilla develops apically. At the time of discharge the zoospores emerge as a miniature cluster, which contains from fifteen to twenty-five spores. Swarming of these spores in a vesicle has never been observed, but this may be due to premature discharge. However, since the miniature cluster is similar in appearance to the cluster which emerges from the primary zoosporangia, it is very probable that the small cluster is enveloped by a vesicle also. Following discharge of zoospores, the small hyaline basal sporangium begins to collapse (fig. 49), and within 8 to 32 hours it has usually completely disappeared.

The resting spores of *P. endogena* have been found to be viable for 3 years when kept in desiccated soil in a metal container. New cultures have been established by placing such desiccated soil in distilled water and adding cockroach integuments. However, 3 or 4 weeks are required before a thriving culture is established from this type of material.

Relationships.—The exact taxonomic position and relationships of *Phlyctorhiza endogena* are not certain. As noted before, the superficial appearance of its thallus is very similar to that of *Rhizophlyctis*. However, the sporangium of *P. endogena* originates intramatrically as an enlargement of a portion of the germ tube or incipient rhizoids and not

vesiculations apparently not continuous with the sporangium. ×1180.—Fig. 32. Empty sporangium with some additional rhizoidal vesiculations continuous with the sporangium, while others are not. ×1180.—Fig. 33. Empty sporangium with rhizoidal vesiculations anastomosing. ×1180.—Fig. 34. Empty sporangium with additional vesiculations arising not only from the rhizoids, but also from the sporangial periphery as well.—×1180.

Fig. 3, 4, 5–8, 37, 39–41, and 45–49 were drawn from living material; all others from material fixed and stained *in toto* with Flemming's tripe dye. Drawings were done with the aid of a Zeiss drawing prism and X 10 compensating oculars and enlarged by means of a pantograph.

Note: Magnifications given for the illustrations in papers I, II, and III of this series [Amer. Jour. Bot. 32 (7, 8); 33(5)] should be reduced to one-half.

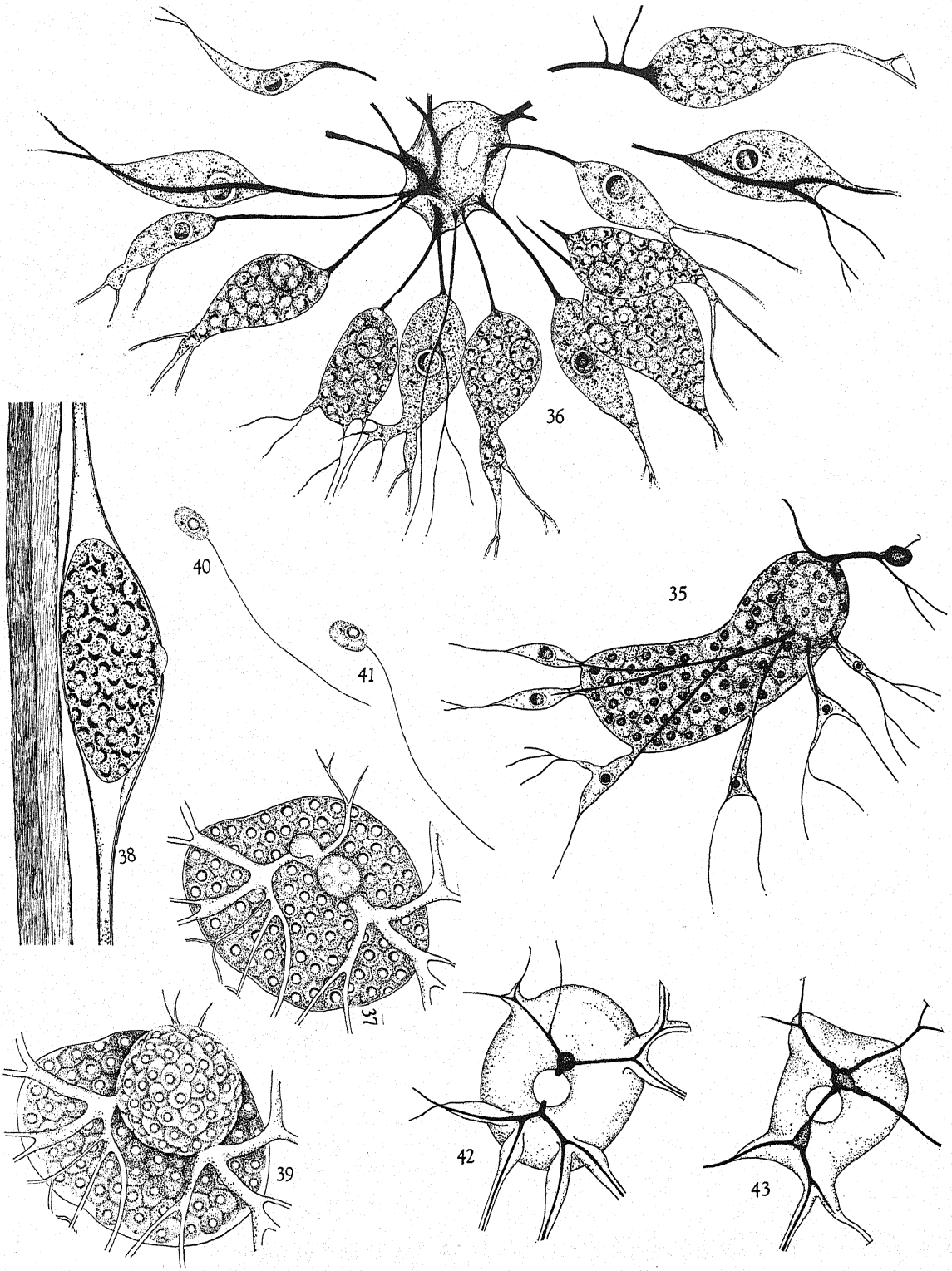


Fig. 35–43.—Fig. 35. Mature sporangium with nucleated rhizoidal vesiculations, three of which are delimited from the sporangium by septa. ×1180.—Fig. 36. Polycentric thallus with an empty primary sporangium and thirteen secondary sporangia in various stages of development. ×1180.—Fig. 37. Mature thallus just before spore discharge. ×1580.—Fig. 38. Cross section of integument showing exit papilla penetrating the outer layer of the basement membrane. ×1180.—Fig. 39. Discharge of zoospores. ×1580.—Fig. 40. Zoospore with centric refractive globule. ×1970.—Fig. 41. Zoospore with lateral refractive globule. ×1970.—Fig. 42. Empty sporangium indicating that part of the

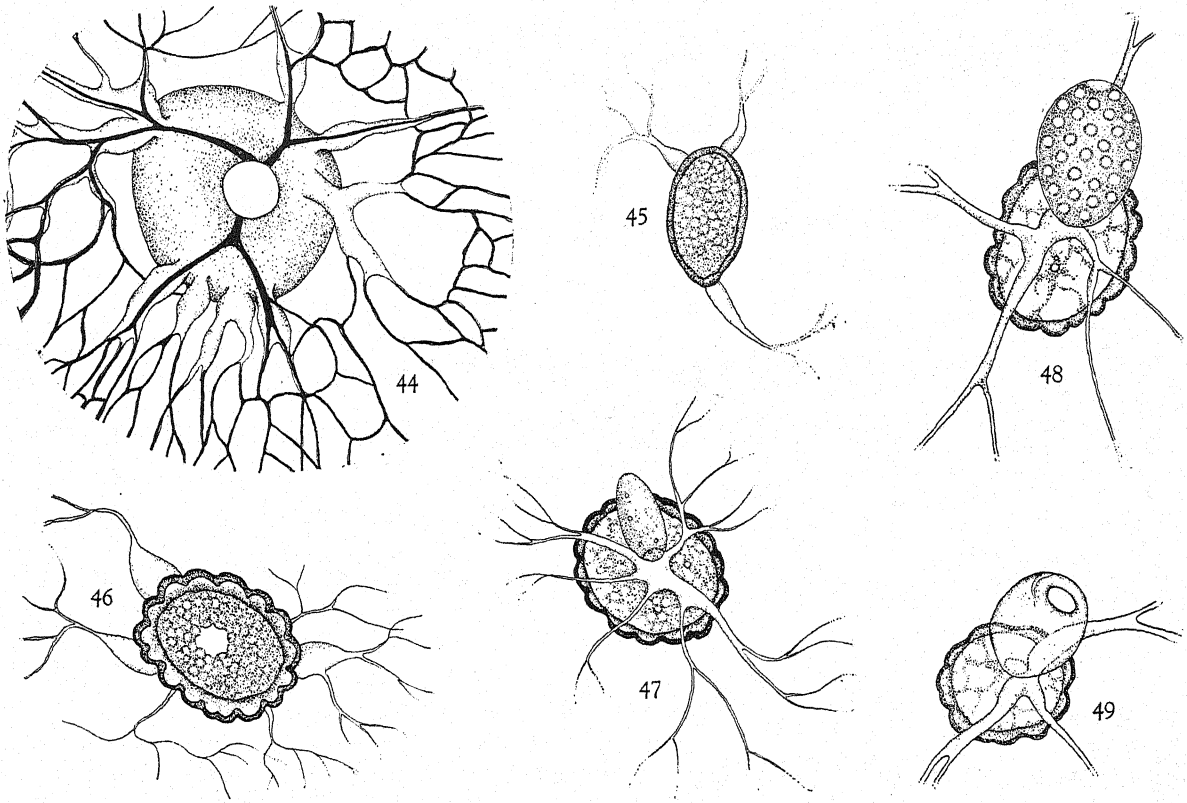


Fig. 44–49.—Fig. 44. Rhizoids terminating smoothly and flush with periphery of the exit papilla. ×1180.—Fig. 45. Dorsal view of smooth walled resting spore. ×1580.—Fig. 46. Dorsal view of tuberculate resting spore with vesiculate rhizoids. ×1580.—Fig. 47. Beginning of germination of the resting spore. Ventral view. ×1580.—Fig. 48. Later stage in germination of the resting spore. Ventral view. ×1580.—Fig. 49. Empty zoosporangium of the resting spore beginning to collapse. ×1580.

directly from the zoospore cyst as is reported to occur in species of *Rhizophlyctis*. For this reason, the two genera do not appear to be closely related, although they are similar in appearance. Because of its type of development, *P. endogena* may be placed provisionally in the family Entophlyctaceae Whiffen (1944) in which the sporangium forms intramatrically from a portion of the germ tube.

SUMMARY

Phlyctorhiza endogena n. gen., n. sp. is saprophytic within the basement membrane of insect integuments. It is predominantly monocentric, but occasionally polycentric. The thalli are characterized by dorso-ventrally compressed sporangia which typically dehisce by a blunt basal exit papilla, and by subsporangial, freely anastomosing rhizoids which may be locally vesiculate. The resting spores are formed asexually, have pigmented smooth, undulate, or tuberculate walls, and in germination function as prosporangia. The fungus has to date been collected in insect integuments floating in water samples from near Lakewood and Franklin Lakes, N. J., and also near Sharon, Conn. It has also been isolated on insect integuments from soil samples collected near Sharon, Conn., and Woods Hole, Mass.

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rhizoidal axis was torn away by the emerging spores. ×1180.—Fig. 43. Part of rhizoidal axis remaining as a shriveled bar extending over the exit orifice following discharge. ×1180.

AN ELECTRON MICROSCOPE STUDY OF TWO STRAINS OF POTATO X VIRUS [1]

William N. Takahashi and T. E. Rawlins

Two STRAINS of potato X virus, namely potato virulent ringspot virus and potato latent mottle virus have been described by Larson (1943, 1944). The above names are those suggested by Larson in recent correspondence.

Our first purification of these viruses was by differential centrifugation as described by Rawlins, Roberts and Utech (1946) for tobacco mosaic. This treatment was followed by filtration through Celite 505. This method of purification, which had given minimum aggregation of tobacco mosaic virus, caused considerable end-to-end aggregation of these strains of potato X virus. Accordingly the method of purification was modified as follows to avoid the aggregation resulting during high speed centrifugation. Nineteen days after inoculation the Turkish tobacco plants were placed in a dark chamber for about 16 hours. Storage for such a period in the dark usually decreases the difficulties in purification. The leaves were harvested, the mid-ribs discarded, and 1 g. of the remaining tissue was finely ground in a ground glass homogenizer. Four cc. of distilled water were added to the ground tissues and the mixture was centrifuged ½ hour at 3000 r.p.m.

The supernatant of the ringspot preparation showed faint stream double refraction which disappeared when a sample was slightly diluted with distilled water. The supernatant of the latent mottle preparation could be diluted 1–12 with distilled water before stream double refraction disappeared. The stream double refraction apparatus used in this work was described by Takahashi and Rawlins (1933).

The undiluted supernatants were then filtered under suction through a layer of Celite 505 about 2.5 mm. thick. After this treatment the ringspot filtrate failed to show detectable stream double refraction whereas the latent mottle filtrate could be diluted 1–2 before stream double refraction disappeared.

The ringspot filtrate was diluted 1–20 and the latent mottle filtrate was diluted 1–50 with distilled water. Drops of these diluted, partially purified viruses were dried on Parlodion films for microscopy. A micrograph of virulent ringspot virus is shown in fig. 1. Micrographs of the latent mottle virus failed to show any detectable difference in the appearance of the particles from those of the ringspot virus. It is evident from the length distribution shown in fig. 2 that the two strains do not show any detectable difference in length of particles. Both differ considerably from the straight, apparently rigid, type of particle characteristic of tobacco mosaic in that these potato viruses apparently show

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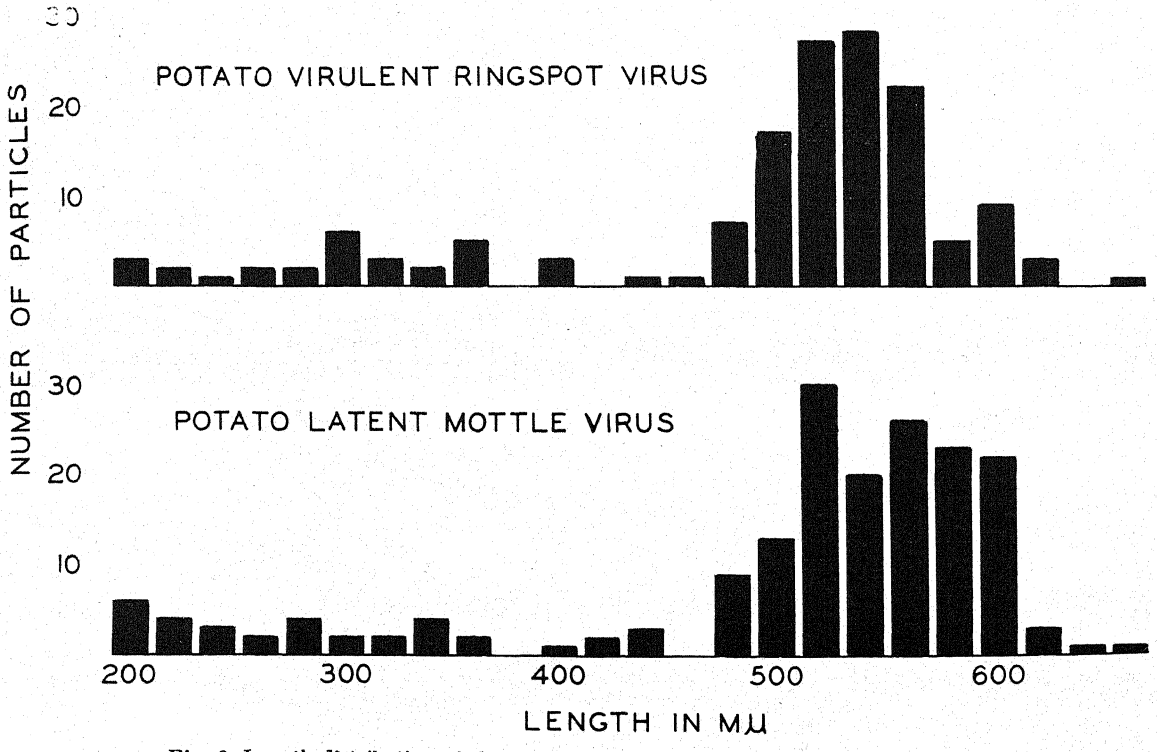


Fig. 2. Length distribution of the particles of the two strains of potato X virus.

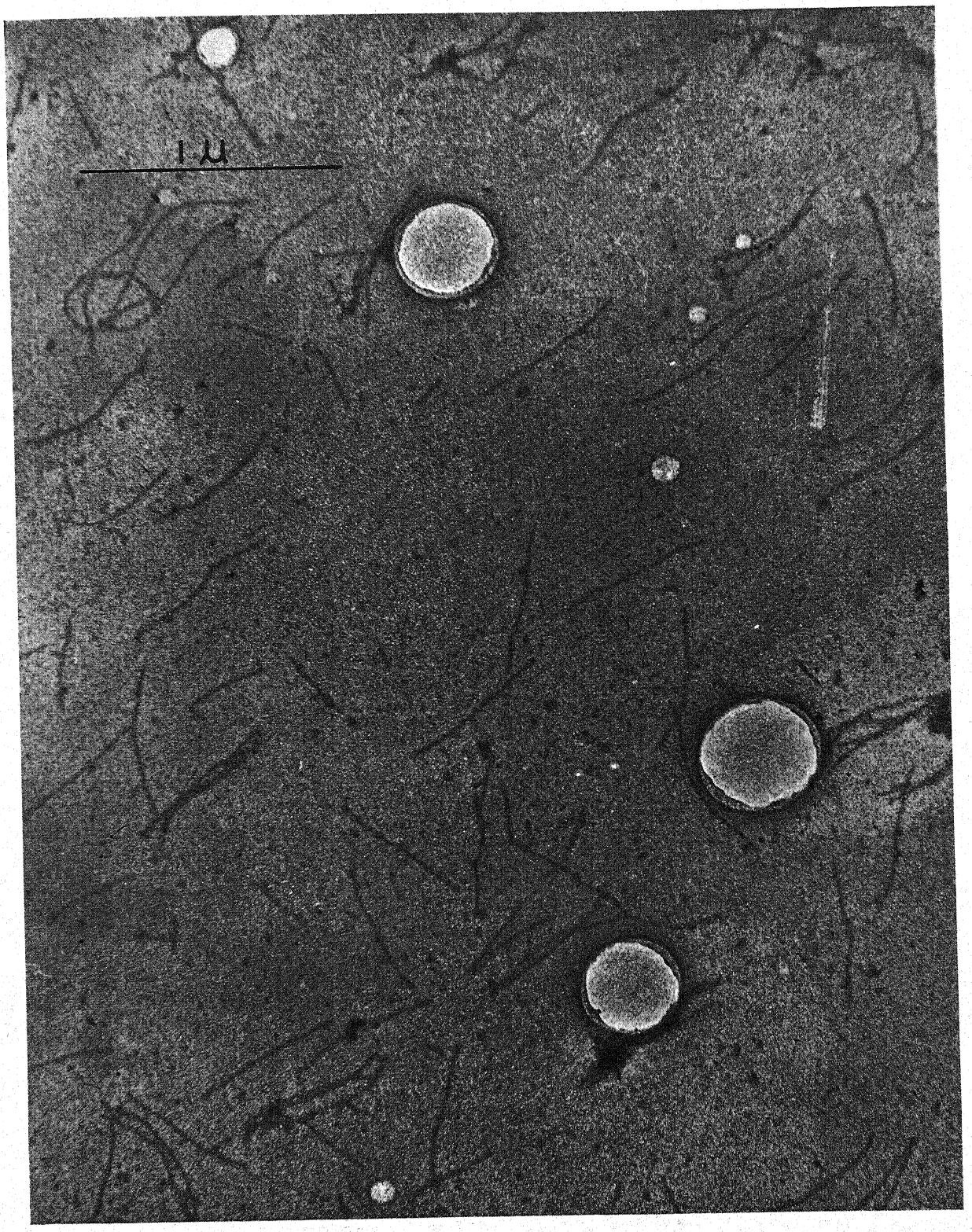


Fig. 1. Micrograph of partially purified potato virulent ringspot virus.

little rigidity and frequently are curled into various forms. This condition makes accurate length measurements difficult. However, it is evident from the length distribution shown in fig. 2 that a high proportion of the particles in each preparation have lengths between 500 and 600 mμ.

Measurements of the width of twenty particles of each virus gave values varying from 14 to 18 mμ for each virus, a mean width of 15.7 mμ for the ringspot virus and 16.1 mμ for the mottle virus. These results indicate that these two viruses do not show a significant difference in width.

It is interesting that although these two strains of potato X virus cause entirely different symptoms on Turkish tobacco they exhibit no detectable difference in the form or size of the virus particles. This is in accordance with the findings of Takahashi and Rawlins (1946) with a yellow mutant of tobacco mosaic virus which was found to have virus particles indistinguishable in form and size from the original tobacco mosaic virus particles.

SUMMARY

Larson's potato virulent ringspot and potato latent mottle viruses were studied with the electron microscope. They were found to be indistinguishable in form and size. A high proportion of the particles have lengths between 500 and 600 mμ. The particles lack the rigidity characteristic of tobacco mosaic virus and are often curled into various forms. The similarity in appearance of the two strains of potato X virus is in agreement with the idea that mutations are usually not accompanied by detectable changes in the appearance of the virus particle.

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ORGANIZATION OF THE SHOOT OF PSEUDOTSUGA TAXIFOLIA (LAMB.) BRITT. I. STRUCTURE OF THE SHOOT APEX [1]

Clarence Sterling[2]

THE SHOOT apices of gymnosperms have recently been the subject of intensive investigation. Most of the studies have agreed substantially on the fact that the shoot apices of the species investigated have a cytohistological zonation in Schüepp's (1926) sense of the term. Thus, there are zones in the shoot apex in which occur differing degrees of mitotic activity, different directions of growth, differences in cell constitution, etc., these zones being combined in various manners. The reader is referred to the paper of Foster (1943) for a discussion of the concept of zonation and for a striking example of zonal structure in a cycad.

It has been noted that zonal structure varies during the growing season in those plants which have a definite growth periodicity. Accounts of apical behavior in shoots of conifers with periodic growth (Allen, 1945; Kemp, 1943; Korody, 1937) seem to indicate that there is a significant difference between this type of apex and that of *Sequoia* (Sterling, 1945). Certain problems of growth, tissue organization, and tissue pattern present themselves in the periodic structure. The present study was initiated to analyze the mode of vascular development within such a seasonal framework. This first paper considers the organization of the shoot apex.

MATERIALS AND METHODS.—The Douglas fir trees used in this study were located in a dense stand above Alpine Dam in Marin County, California. This area has since been partially burned over. Biweekly collections of shoot apices were made from February 1941 to January 1942. All the apices came from the leading shoots of vigorous young plants, 1 to 3 meters tall. Several killing reagents were used, of which the most satisfactory was the modified Navaschin fluid of Randolph as given by Johansen (1940). The apices were dehydrated and embedded according to the technique outlined by Ball (1941a). Sections were cut 10 μ thick and stained with a fast green variation of Foster's (1934) method.

PERIODICITY OF THE SHOOT APEX.—The dormant bud (fig. 12, 17, 22) begins to expand in February, pushing apart the bud scales and eventually emerging in March. The leaves elongate rapidly by the activity of a rib meristem as does the shoot. The shoot apex above the leaf primordia slowly in-

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[2] The writer wishes to express his thanks for the use of the facilities of the University of California and of the University of Illinois in this study. He is indebted to Dr. A. S. Foster for a critical reading of the manuscript.

creases in size (compare fig. 13 and 17). In March, when the young shoot has elongated to about four times its original length, its apex begins to lay down a series of bud scale primordia (fig. 3, 13). During bud scale deposition, which lasts about five weeks, the size of the apex increases very slowly. Cataphyll formation ends just previous to the so-called "grand period" of growth of the elongating shoot.

About the middle of April, when all the cataphylls for the winter bud have been initiated, the shoot apex begins to grow rapidly (fig. 5, 14), principally by mitotic activity. As the apex enlarges, leaf primordia begin to develop acropetally along the flanks of the apical cone (fig. 6, 15, 20). These primordia occur well below the cone summit, so that the leafless portion of the shoot apex is of considerable extent at this time. With certain sequences of meristem zonation and activity which are described below, this development lasts until the middle of September. The embryonic shoot reaches its full length about this time, following which the leaf primordia develop all the way up the cone, leaving only a fractional portion of it free as the shoot apex meristem. The dormant bud is completely formed during October. The series of form changes and structural development throughout the growing season is represented in fig. 1–12.

ORGANIZATION OF THE SHOOT APEX.—Allen (1945) has shown that the shoot apex of *Pseudotsuga* is characterized by an apical initial group dividing anticlinally and periclinally at the summit (as in fig. 15, 17, 18 of the present paper). This gives rise to a central mother cell zone below, in which the cells (and their nuclei) enlarge, become lighter staining, and acquire interstitial thickening. A zone of eumeristem is produced from the central mother cells, giving rise peripherally to leaf primordia, cortex, procambium, and epidermis and basally to the pith mother cells (Allen's rib meristem zone). The figure of *Pseudotsuga Douglasii* by Lewis and Dowding (1924) shows clearly the relationship between central mother cells and eumeristem in the dormant bud.

The expanding bud.—From the period of dormancy to the time of bud scale initiation, the shoot apex is borne upward more or less passively, with a slight increase in size as noted above, on the tip of the elongating shoot. The aspect of the apex is principally the same as that of the dormant bud (fig. 17) during this time. The central mother cells are only slightly larger than the cells of the short eumeristem zone below; they are distinguished from the latter principally by their lightly-staining quality and their thicker walls. Just previous to cataphyll formation, the apex becomes somewhat steeper (*i.e.*, more conical) from the originally more hemispherical form of the resting bud (fig. 13).

It should be noted that the shoot apex does not at this time contribute tissue to the expanding shoot below. It undergoes little noticeable meristematic activity. The cells of the pith, which are isodiametric in the winter bud, elongate and divide almost simultaneously throughout the shoot. The cells at the base divide somewhat earlier than those nearer the tip of the shoot but not appreciably so. The cells extend and divide continuously to build up series of rib meristem files. In this manner, the whole pith region of the young shoot constitutes essentially an active rib meristem. Relatively early, the cells at the base of the pith (above the inactive pith and crown of the previous season) develop much longer files of rib meristem lineages than do those nearer the summit. This is in agreement with the discussion by Jost (1913) on the behavior of the spruce bud: "Bei der ferneren Streckung bildet sich aber eine Zone maximalen Wachstums aus; diese liegt zuerst an der Basis der Sprosses und rückt allmählich nach der Spitze vor." The activity of the rib meristem decreases acropetally, and the cells of the pith directly below the shoot apex merely elongate without dividing (fig. 13).

Cataphyll initiation.—During bud scale deposition, the shoot apex enlarges to about twice the height (above the leaf primordia) of the dormant apex, becoming proportionately narrower (fig. 4). The eumeristem area becomes slightly more extensive in depth and much deeper-staining than previously. The central mother cell zone still does not appear very conspicuous at the time of this activity: it is only slightly larger than at dormancy.

As the apex enlarges, the cataphylls are produced in rather rapid succession in a rosette arrangement. These elongate almost as quickly as they are formed. They soon overarch the summit of the apex due to marked abaxial elongation and vacuolation, especially at their tips. At this stage, there is no detectable histological difference between the outer scales, which will become coriaceous, and the inner ones, which will be scarious in texture. All the cataphylls are provided with procambial strands which extend into their bases (fig. 16).

Toward the end of this period, the cells of the pith which have been laid down simultaneously with the cataphyll primordia begin to expand transversely (fig. 4, 14). The vascular strands are bowed outwardly by the expansion, which goes on continuously until about July. As they enlarge, the pith cells also undergo longitudinal and oblique divisions, so that the number of vertical cell files in this region is increased. The individual cells soon become markedly tabular in shape.

Below this zone, sclereids develop randomly in the pith of the elongating shoot. The pith cells in this region also enlarge somewhat and occasionally divide by longitudinal walls to accommodate to the increase in pith diameter above. Intercellular spaces are abundant as the cells round off. The sclereids are most numerous just below the zone of transverse expansion but also occur scattered at greater depths in the pith. Their walls acquire definitive thickenings rapidly. A more detailed discussion of sclereid ontogeny will appear in a subsequent paper.

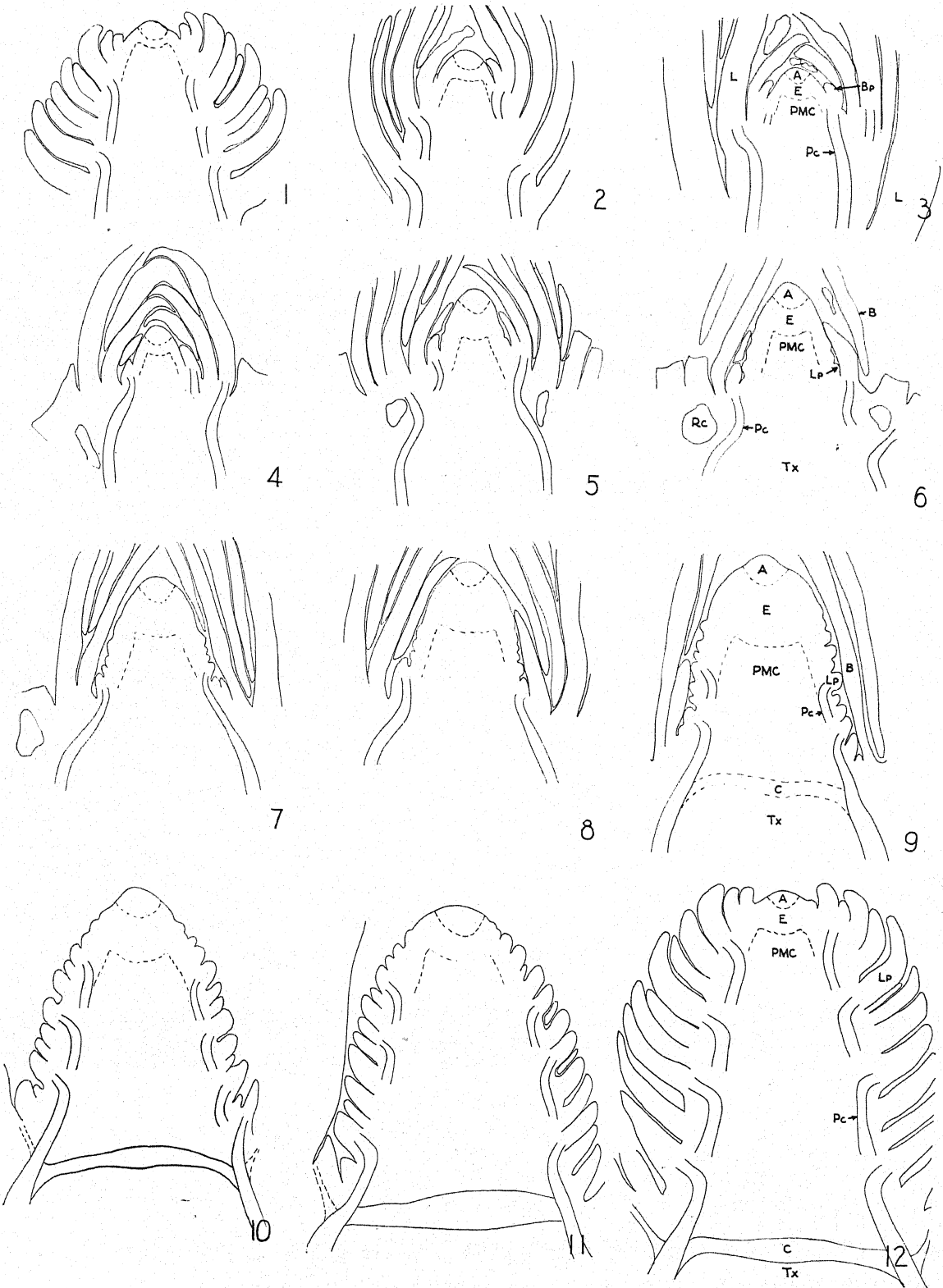


Fig. 1–12. Micro-projection drawings of median longisections of the shoot apex throughout the growing season.—Fig. 1. Shoot starting to elongate. Feb. 22.—Fig. 2. More extensive elongation of shoot and leaves. Mar. 2.—Fig. 3. Initiation of bud scale primordia. Mar. 9.—Fig. 4. Active bud scale formation and apical enlargement. Beginning of transverse expansion in the pith. Apr. 6.—Fig. 5. End of bud scale formation and beginning of marked apex

Coinciding with cataphyll formation, a localized growth activity is initiated in the cortex. This activity is due to the formation of a cambium-like "receptacular meristem" which arises at the inner boundary of the cortex under the newly-formed bud scales. This meristematic plate, which can best be seen in the non-vascularized regions between pith and cortex (fig. 16), is parallel in orientation to the vascular strands of the shoot. It adds derivatives outwardly by tangential divisions.

The products of the divisions soon elongate perpendicularly to the plane of the meristem, serving to raise the bud scales. Thus long cell files, parallel to one another, are soon distinguishable in the cortex. The combined effects of pith expansion, meristematic activity, and cell elongation in the cortex raise the bud scales to such an extent that they appear borne on a receptacular structure, as noted by Korody (1937) and Kemp (1913). The organization of the receptacular meristem recalls that of the thickening meristem in certain monocots (Ball, 1941b; Helm, 1936) and in cycads (Foster, 1939; 1941; 1943). The cell files which elongate tend to push apart, so that this area of the cortex becomes somewhat lacunate. At this time sclereids of a particularly large size (fig. 14) are seen to develop in this tissue.

Just outside the vascular strands in the shoot axis and parallel to them, certain cells in the cortex can be seen to enlarge in diameter and become very long. Many were well over 1000 μ in length. Each cell appears generally to have a single nucleus, which is quite large (fig. 19), and occasionally two nuclei were found in a cell. The nuclei disintegrate early, at about the time the cells become filled with a large quantity of granular ergastic matter. Busse (1893) has called attention to these "Gerbstoffzellen" which soon become "Gerbstoffschläuche." They may be related to the secretory elements found hypodermally and in the stele of embryos of *Larix* (Schopf, 1943) and *Pseudotsuga* (Allen, 1945), although much larger than these.

Formation of leaf primordia.—When all the bud scale primordia have been formed, the shoot apex begins to enlarge rather rapidly (fig. 5, 14). The apex does not form any primordia at first. It rises as an elongated naked cone for some distance before leaf primordia are initiated on its flanks.

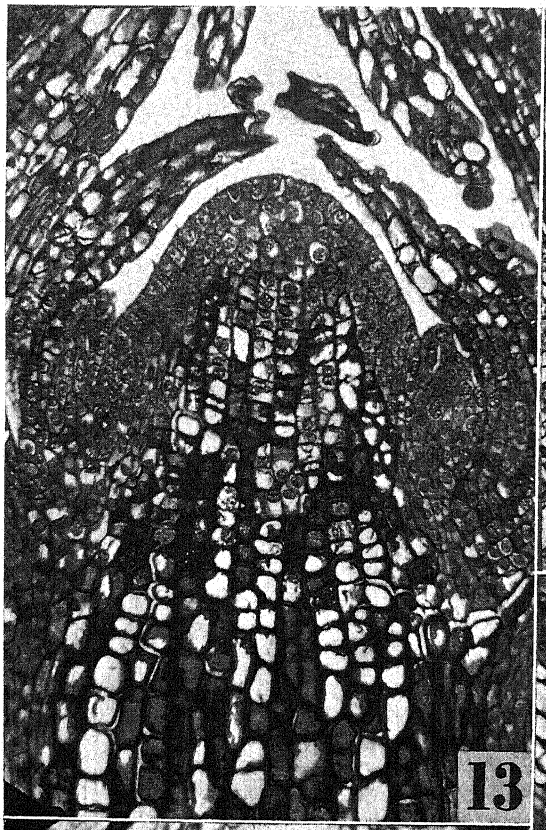
a. The conical apex.—During leaf production, the shoot apex assumes certain characteristic shapes. When it first enlarges during this period, it is conical or roughly parabolic (fig. 5–7, 15, 20). The central mother cell zone increases in size and becomes quite prominent (fig. 15). Its cells increase in volume, become very light-staining, and acquire heavy corner thickenings. Also the eumeristem zone experiences a size increase, so that it is notably more extensive than during the period of cataphyll formation. There is no marked rib meristem activity at this time. The slightly more vacuolate cells below the eumeristem may be termed the pith mother cells since it is from them that the succeeding year's pith will take its origin. These pith mother cells are formed principally by transverse divisions in the eumeristem. Consequently, although they are laid down in long files similar to those produced by typical rib meristem growth, these cells experience relatively few subsequent divisions at this stage.

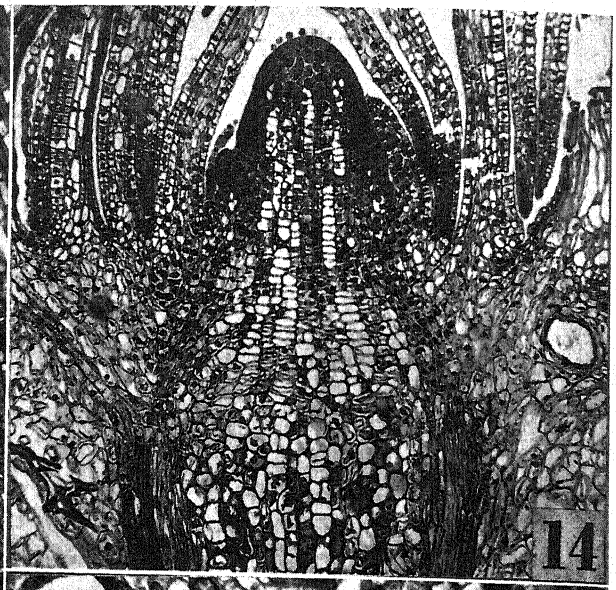
Leaf primordia, as indicated above, are produced on the lower flanks of the enlarged conical apex, but there is no immediate extension of the primordia. They remain as small meristematic humps on the surface of the cone and are provided with procambial strands. Immediately below the meristematic cone, the vacuolate cells of the pith expansion zone can be seen extending actively in the transverse direction.

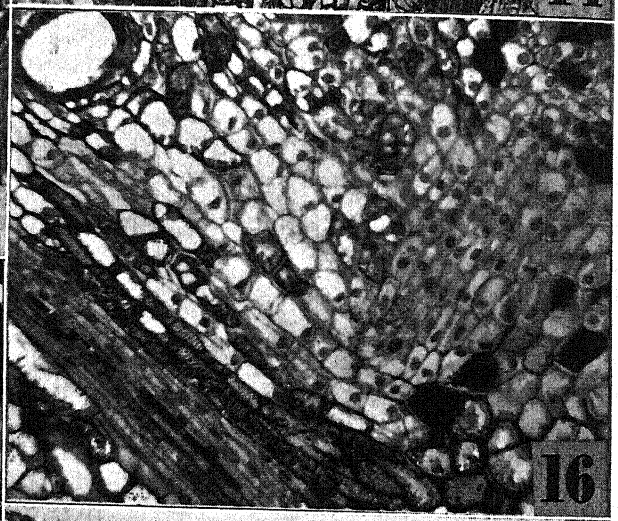
b. The mamillary apex.—When the enlarged apex has attained about one half or two thirds of its future length, a new activity is initiated in the leafless portion of the apical cone. The cells of the eumeristem divide very actively, so that the contrast in size to the central mother cells is even more marked than previously (fig. 18). This condition recalls the zonation of *Ginkgo* (Foster, 1938), the cycads (discussed by Foster, 1943, and Johnson, 1944), and *Sequoia* (Sterling, 1945). The division walls of the eumeristem are oriented periclinally and anticlinally with respect to the lower boundary of the central mother cell zone. The eumeristem zone is thus increased to about thirty cells in depth. Simultaneously, the transverse diameter is also increased so greatly that shoulders of this tissue project below the central mother cell area as hump-like expansions of the apex. (Compare Louis', 1935, foliar buttresses.) The shoot apex now has a mamillary aspect, quite similar to the appearance of a conifer embryo just prior to the formation of cotyledonary primordia (fig. 9, 18, 21).

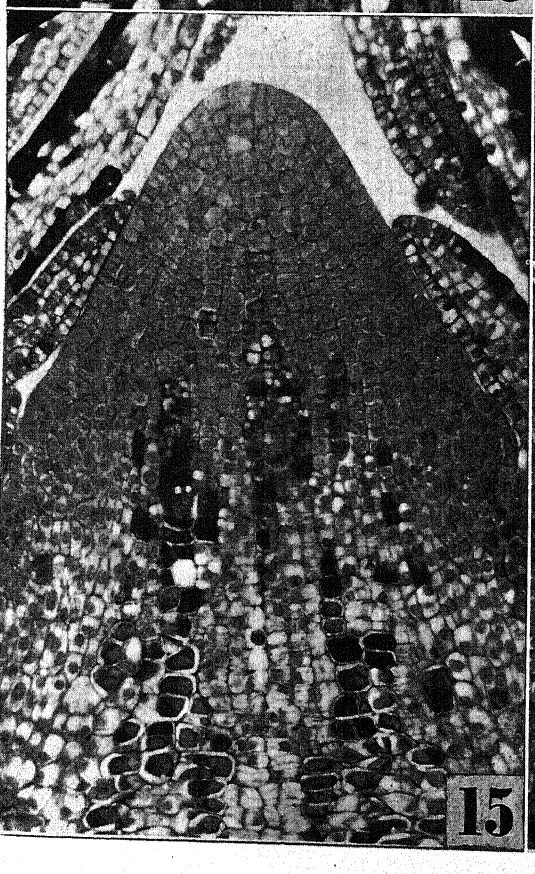
As the cells produced by the eumeristem enlarge, the apex is increased in length (fig. 10, 11). Once

enlargement and formation of leaf primordia. Apr. 20.—Fig. 6. Bud scales almost mature. Apex enlarging and forming more leaf primordia. May 12.—Fig. 7. Augmentation of apex size, increasing depth of eumeristem zone, and bowing of procambial strands due to active expansion of pith region. May 18.—Fig. 8. Eumeristem zone increasing in depth and beginning to extend transversely. June 29.—Fig. 9. Stage of mamillary apex. Walls of cells in future crown region beginning to acquire (in some apices) a slight thickening at this stage. July 10.—Fig. 10. Young shoot experiencing rapid leaf formation and axis elongation. Walls of crown cells thickening noticeably. Aug. 17.—Fig. 11. Near dormant apex. Eumeristem zone decreased in extent and almost all leaf primordia formed. Sept. 21.—Fig. 12. Dormant bud. Apex reduced in size and extent of all but pith mother cell zones. Crown area extending in slight development through cortex. Jan. 18. All fig. ×29. Legend: A, the apical initial-central mother cell complex; B, bud scale; Bp, bud scale primordium; C, the crown; E, eumeristem tissue; L, leaf; Lp, leaf primordium; Pc, procambium; PMC, the pith mother cells; Rc, resin cavity; and Tx, the zone of transverse expansion.


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the shoulders have been formed, leaf primordia differentiate in close order up the apical cone, leaving only a fractional portion of the apex free. The differentiation of primordia and pith mother cells "uses up" most of the extensive zone of eumeristem. The central mother cell zone diminishes somewhat in the free shoot apex, and its cells decrease in size.

At this time, the walls of about six to fifteen horizontal layers of the uppermost transversely expanded cells of the pith (just below the pith mother cells of the new bud) begin to thicken. This is the zone of cells which constitutes the "crown" noted by various workers in the dormant conifer bud. In Douglas fir there is a trace of this zone in the cortex (fig. 10–12, 22), extending to the base of the youngest bud scale. It is not well-developed, however, and neither the amount of wall-thickening nor the number of cell layers involved is equivalent to that of the pith. The structure of the walls (pitting, thickness, etc.) and the ergastic contents of the cells agree substantially with that reported by other workers.

The dormant bud.—When the dormant bud has been fully formed, it is about 2000 μ in height above the crown of the old pith (fig. 12, 22). Its own pith consists of relatively small, isodiametric cells, which stain more lightly than the eumeristem but not markedly so. The cells are arranged in long vertical series, but there are few coherent files of daughter cell groups as in typical rib meristem. Throughout the pith are found cells filled with deeply-staining ergastic material. These cells are noticeable as soon as the apex begins to expand for the production of leaf primordia. However, their contents should not be interpreted as indicating a cessation of activity on the part of those cells. A protoplast is present in each of these, and as the apex enlarges during leaf primordia formation, the cells originally filled with this material give it up and other cells acquire that type of content. At initial bud expansion in the spring, no ergastic-containing cells are to be found anywhere in the pith. The cells in the pith of the dormant bud are mainly of the pith mother cell type.

At the summit of the embryonic shoot the zones of meristem are readily recognizable. However, the apical initial-central mother cell area is of small extent and is not set off very sharply from the eumeristem zone below (fig. 17). The eumeristem grades into the pith mother cell area in the center of the shoot; it is relatively shallow in depth as compared to its extent during the period of active growth. The leaf primordia, all supplied with procambial strands, decrease in size acropetally from the long leaves at the base of the bud to the few-celled humps at the summit. The bundles to the primordia have few differentiated vascular elements. However, the same bundles below the crown (which is penetrated by all the strands) have extensive tracts of mature xylem and phloem tissue. This, it will be recalled, is the same situation that exists in the winter bud of *Sequoia* (Sterling, 1945), in which however the crown area is not as precisely defined as in *Pseudotsuga*.

Below the crown of the dormant bud is found a highly lacunate tissue (fig. 22), but this tissue is not torn and disintegrated as in *Torreya* (Kemp, 1943). Both pith and cortex have this lacunate zone, which is a much expanded region of the shoot. The pith and cortex are wider at this point due to the developments outlined earlier. Below this zone occurs a region of about equal vertical extent, in which the pith narrows and in which are to be found abundant sclereids, branched in all planes and very thick-walled. As Allen (1945) has pointed out, there are no sclerotic diaphragms in the pith of *Pseudotsuga*, contrary to the report of Lewis and Dowding (1924).

Discussion.—One of the salient facts about the *Pseudotsuga* shoot apex and that of other Abietineae and *Torreya* is the remarkable type of seasonal behavior. In these plants there is no long continual period of open growth as in *Sequoia*. Although the shoot apex exhibits perpetual embryogeny, that embryogeny is periodic or episodic. The production of foliar primordia has become restricted to a definite phase of annual shoot development and takes place with a definite seasonal rhythm. Comparable periodic phenomena occur commonly in many angiosperms.

The specialization displayed in periodicity of structure is not only external. It is also expressed in the histological development of the shoot. In *Sequoia* (Sterling, 1945), continuous embryogeny produces a topographic sequence, in active shoots, of apical initials, central mother cell zone, eumeristem, pith mother cells, and rib meristem. These zones remain in approximately the same proportions to each other throughout the growing season. During the period of approach of dormancy, the only significant change in the redwood apex is the progressive encroachment of the rib meristem zone on the pith mother cell area.

However, in Douglas fir the aspect is quite different from that of redwood. In its apex, all the pith mother cells are formed one season, from which an active rib meristem develops the next. In other words, there is almost a complete chronological

Fig. 13–17. Median longisections through the shoot apex.—Fig. 13. Beginning of formation of bud scale primordia. Pith of expanding shoot ends about the level of insertion of the new primordia. ×150.—Fig. 14. End of bud scale formation. A large sclereid is visible in the cortex. Resin cavity can be seen at right in cortex. ×68.—Fig. 15. Enlarging conical apex with a few leaf primordia. Note contrast between central mother cell zone and eumeristem and between pith mother cells and zone of transverse expansion. ×150.—Fig. 16. Region of "receptacular meristem" showing files of cells being cut off and elongating under the bud scales. Resin cavity is seen at upper left. At lower left is a portion of a vascular strand to a bud scale. ×150.—Fig. 17. Apex of dormant bud. Note small amount of eumeristem. The files of cells in the pith have not been produced by rib meristem activity despite similarity. ×150.

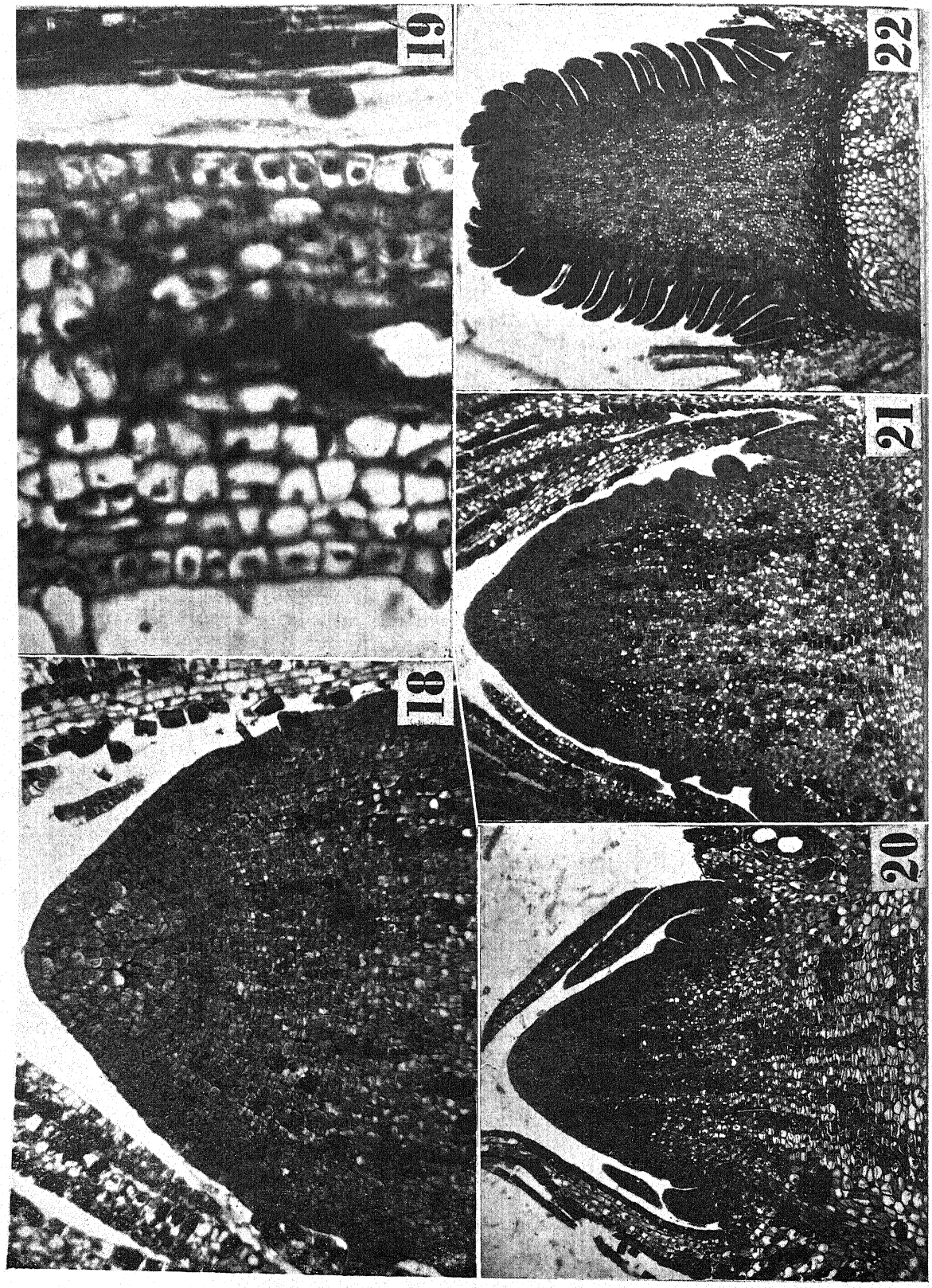


Fig. 18–22. Median longisections of the shoot apex.—Fig. 18. Mamillary apex, showing sharp demarcation between central mother cell zone and eumeristem. ×150.—Fig. 19. Secretory cell to left of procambial strand. Note size of

transference of the topographical relations that are found in redwood. This is true, incidentally, not only for the relationship between pith mother cells and rib meristem, but it stands, in a broader sense, for growth primarily by cell division as counterposed to growth mainly by cell elongation. The formation of leaf primordia stands in the same relation to their elongation. The transposition of the relationship between organ elaboration and organ formation from space to time seems fundamental in the periodicity of the shoot of the Abietineae (Allen, 1945; Busse, 1893; Korody, 1937), *Torreya* (Kemp, 1943), and various angiosperms (Askenasy, 1877; Schüepp, 1929; and others).

Sachs (1893) recognized this fact, amending his classical formulation of the three phases of growth in an organ. In this revised scheme, he added a fourth phase which preceded the other three, namely: the determination of the number and position of the embryonic organs (*i.e.,* appendages). He then arranged these four phases into two developmental periods: the determination of organ number and position and the embryonic growth of these (mitotic activity) constitute a *morphological* period. This is contrasted with the *physiological* period, which embraces cell elongation and tissue maturation. The two periods can occur at different times, separated by a period of dormancy. Thus, in telescoped buds like those of Douglas fir, the embryonic shoot has passed through its morphological period. The physiological period is expressed in the following season. Went (1944), in a study of tomato development, substantiated and extended Sachs' interpretation.

The present study confirms Kemp's (1943) observation on the relationship between apical zonation and shoot periodicity in *Torreya*. She states, "This zonal pattern is reduced to a minimum in periods of inactivity and attains a maximum degree of clarity during the formation of appendages." In *Pseudotsuga* not only is the zonal structure most distinct during the period of foliage primordia initiation, but also the individual zones are of maximum size in this phase of development. On the whole, there is a noteworthy resemblance in the structure of the bud and general shoot development between *Torreya* and the members of the Abietineae which have been studied so far, except for *Pinus* (Korody, 1937).

The existence of procambial strands to the meristematic leaf primordia as well as to the bud scales should be stressed. Barthelmess (1935) called attention to the fact that he found a bud of Douglas fir in which a ring of vascular tissue was differentiated although present below the bud scales, which were said to have "keinerlei Bündel." However, the extensive survey of Schumann (1889) and the results of the present study contradict those observations. The large number of transections and longisections of the writer show definitely that all bud scales in *Pseudotsuga* are provided with vascular bundles (up to their bases at least) and that these bundles have definite xylem and phloem elements (see fig. 16). Barthelmess' fig. 6, which purports to show the last leaf trace "entering" the stelar cylinder, actually corresponds to sections of the writer through the zone of transverse expansion of a near-dormant bud, in which cataphyll traces are "entering" the vascular cylinder. Since no such shoot as Barthelmess describes was found, the conclusions drawn from his basic premise therefore appear to be in doubt. It is unfortunate that Wetmore (1943) used this report as one of the bases of his evaluation of leaf and shoot relationships.

The work of Schröder (1869) on maple has been discussed by Busse (1893) and Korody (1937). Both compare his "Markkuppe" to the crown of the coniferous bud and mention the fact that the "Markzwischenstück" (a lacunate mass of roundish pith cells) occurs above the pith cap in maple and below it in conifers. However, the pith cap and conifer crown may not be comparable: Schröder goes on to describe, above the lacunar area, a tissue of collenchymatously thickened cells at the base of the young pith. If any analogy is to be drawn, the above-mentioned tissue might be more like the abietinous crown in position and structure.

A further consequence of the episodic behavior of the shoot in *Pseudotsuga* should be indicated. During the season's growth, the shoot produces from three to five axillary buds near the tip, spaced so closely together as to appear whorled. These buds are constant in occurrence and position. It is immediately apparent that a study of the vascular development in an apex which produces its axillary buds in a uniform pattern may be expected to reveal some definite relations of axillary bud formation. According to Miller and Wetmore (1946) a meristematic bud primordium is to be found in the axil of each leaf of *Phlox Drummondii*. If an adequate histological technique is employed, that species should provide useful material for studying the problem of bud ontogeny and its vascular relations.

SUMMARY

The shoot apex of *Pseudotsuga taxifolia* is characterized by a growth periodicity. Elongation of the dormant bud takes place with very little apical meristematic activity; it is accomplished principally by the formation of rib meristem from the rows of pith mother cells laid down the previous season. Bud scale formation begins about a month after the start of shoot expansion and lasts for a period of about five weeks. The bud scales are

its nucleus in relation to nuclei of cortical parenchyma. A resin canal is seen in the cortex. Unicellular trichomes appear on the epidermis at the extreme left. ×300.—Fig. 20. Conical apex of about same development as that of fig. 6. ×68.—Fig. 21. Mamillary apex similar to one in fig. 18. ×68.—Fig. 22. Dormant bud. Crown area is deeply stained and can be seen partially differentiated in the cortex. ×22.

raised about the shoot apex by the combined effects of pith expansion, meristematic activity in the cortex, and the elongation of the products of this meristematic growth. All the bud scales are provided with vascular bundles.

Following bud scale deposition, the shoot apex enlarges markedly. During this enlargement, leaf primordia for the following season are formed along the flanks of the apical cone. Due to changing distributions of growth patterns and zonal activity, the shape of the apex becomes conical and then mamillary before leaf primordia develop up to the summit. In the fully formed dormant bud, there is a thick-walled crown tissue separating the newly formed bud from the rest of the shoot, except for its vascular connections. The bundles of the leaf primordia have few differentiated vascular elements in the dormant bud.

The zonal pattern of the shot apex varies during the growing season. The zones are most extensive and well-marked during the formation of the leaf primordia. They are least prominent in dormancy and during shoot elongation in the spring. The zones present in Douglas fir are: the apical initial group, giving rise to the ginkgoid central mother cell area, the eumeristem which differentiates from the latter, and the pith mother cells developing basally from the eumeristem in the center of the shoot.

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KERATINOPHILIC CHYTRIDS. I. RHIZOPHYDIUM KERATINOPHILUM N. SP., A SAPROPHYTE ISOLATED ON HUMAN HAIR, AND ITS PARASITE, PHLYCTIDIUM MYCETOPHAGUM N. SP.[1]

John S. Karling

In a previous publication the author (1945) described the technique of using strips of purified chitin as bait for isolating and chitin agar media for culturing chytrids which normally inhabit the exuviae and skeletons of insects in fresh water. Such chytrids were described as chitinophilic because they are chitin loving and appear to derive part or most of their nourishment from chitin.

The present paper introduces the use of keratinized tissues of animals as a different type of substratum for isolating and growing chytrids. The use of these substrata as bait in soil and water cultures has resulted in the discovery of several new specialized species which will be described in the present and subsequent publications. Inasmuch as these species appear to be limited in occurrence and growth to tissues which contain *keratin* they will be described as keratinophilic chytrids. A large number of fungi, particularly those which cause mycoses in animals, grow well on keratinized tissues, but these species are not very specialized in their nutrient requirements and will flourish on other types of substrata and agar media. Therefore, they are not regarded as strictly keratinophilic. Accordingly, this descriptive term will be used only for species which appear to be unable to grow on any but keratinized substrata or synthetic media which contain *keratin*.

To be of much value for microscopic study of minute fungi like most of the chytrids, a substratum must be very thin and relatively transparent. From these standpoints, human skin has proven to be the most favorable keratinized substratum used so far, although all keratinophilic species do not flourish on it. It may be peeled off in thin layers following severe sunburn and readily stored for future use. Such layers of the outer epidermis are sufficiently transparent and thin to transmit light for adequate microscopic observation and also provide sufficient nourishment for the chytrids to be studied. The "down" of ducks and geese, pinnae of other minute feathers, and fine shreds of wool are likewise favorable. Shavings of nails, hoofs and horns provide nourishment, but they are usually too thick and hard for use in microscopic studies. Hair of babies and other immature animals is unusually favorable because of its fineness and relative transparency. To insure success, all of these substrata should be cut up into small pieces and floated on the surface of the water and soil cultures to be tested. If keratinophilic chytrids are present, they will appear on the substrata within a few days or weeks. Other larger fungi like *Pythium, Saprolegnia, Aphanomyces, Rhizopus,* etc., will probably infect the substrata also if they are present in the cultures, but these species may be distinguished readily from chytrids.

It is not yet certain what are the basic and special nutrients in these substrata which the strictly keratinophilic species require and utilize. It is to be noted, however, that tissues such as skin, finger and toe nails, hair, hoofs, horns and feathers contain varying amounts of *keratin* which upon decomposition yield *cystine* ($C_6H_{12}O_4H_2S_2$). Cystine is the principal sulfur-containing substance obtained from decomposition of proteins and has been shown to be *di (β-thio-d-amino-propionic acid)*. It occurs in small amounts in normal urine and is greatly increased in quantity under certain pathological conditions. Preliminary attempts have been made to grow keratinophilic chytrids on agar media to which *keratin* or *cystine* were added, and although these cultural experiments have not been successful, it is not improbable that strictly keratinophilic fungi utilize *cystine* as one of their basic amino acids.

The present study concerns primarily a species of *Rhizophydium* which occurred in water and soil collected from a small stream in an abandoned and heavily eroded cemetery near Porto Velho, Amazonas, Brazil. The soil and water sample was brought to New York City, diluted with animal charcoal water, and baited with bits of human hair. Within a few days this substratum became heavily infected with the chytrid in question. Later, bits of snake skin, feathers, camel, dog, cow and horse hair, shreds of wool, and shavings of nails, hoofs and horns were added to the culture and soon became infected. Up to the present time this chytrid has grown vigorously on all keratinized substrata available except human skin.

Inasmuch as it was found first in soil from a heavily eroded cemetery where keratin-containing tissues of humans were presumed to be abundant, soil from cemeteries in New York City was collected and tested to determine whether or not the fungus is limited to such localities. All collections yielded an abundance of the chytrid, and it was assumed accordingly, that this species is a common inhabitant of cemeteries. However, more extensive collections and tests showed that it occurs fairly commonly in soil from wood and pasture lands and is not limited to any particular type of locality. So far, in addition to Brazil and New York City, it has

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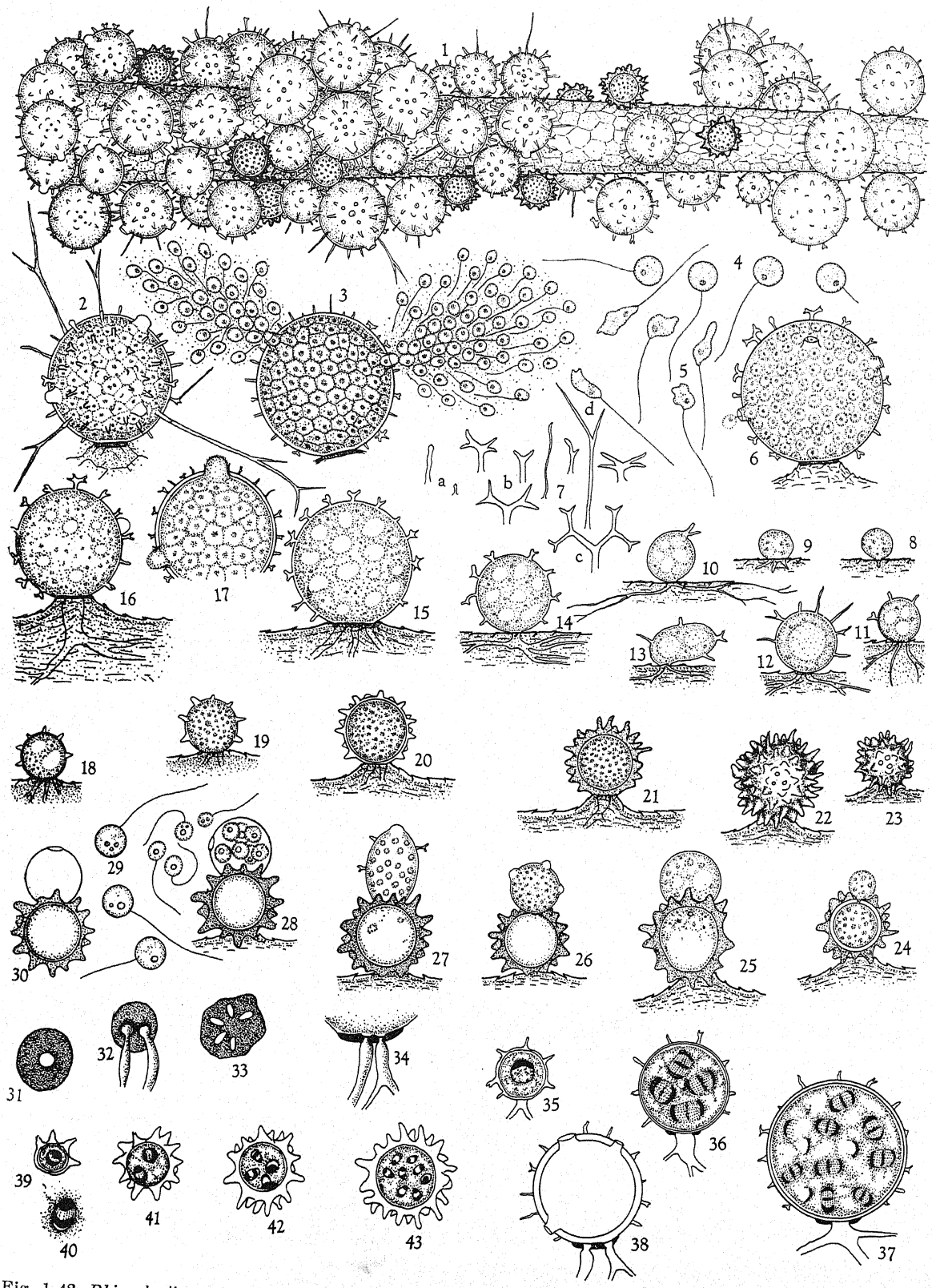


Fig. 1–43. *Rhizophydium keratinophilum.*—Fig. 1. Piece of human hair infected with sporangia and resting spores of *R. keratinophilum.* ×1200.—Fig. 2. Mature dislodged sporangium with basal plate, three exit papillae, and hairs. ×1400.—Fig. 3. Discharge of zoospores from dislodged sporangium. ×1400.—Fig. 4, 5. Active motile and amoeboid

been isolated from soil from Dublin, Ireland, Groeningen, Holland, New Orleans, La., tidewater Virginia, Woods Hole, Mass., Pawling, New York City, and Alden, N. Y., Brookfield, Conn., and Bergen County, N. J. Apparently, it is a common inhabitant of soil which contains bits of keratinized material.

Although this chytrid is widely distributed and grows on most keratinized tissues, all efforts to culture it on the commonly-used agar media have failed. Furthermore, special agar media to which varying amounts of *cystine* were added proved unfavorable also. Likewise, media containing hydrolysates of human hair and pig skin have been tried without success. These preliminary tests indicate that the nutritional requirements of this chytrid may be quite specialized. However, once a favorable medium has been developed, this species should not be hard to culture. Although it is monocentric and produces only one thallus from each zoospore, it grows so vigorously and forms such an abundance of sporangia and zoospores that there is no scarcity of material for inoculation purposes.

Structurally, this species is characterized by predominantly spherical sporangia which are ornamented by short, simple, bifurcate or dichotomously branched spines, or long fine, simple or branched hairs, spherical zoospores containing a minute, hyaline refringent globule, and by small, light-brown, prominently-warted resting spores. Accordingly, it differs morphologically from any of the other known species of *Rhizophydium*. Because of these differences and the fact that it occurs only on keratinized substrata in nature, it is diagnosed as a new species and named *R. keratinophilum*.

Rhizophydium keratinophilum n. sp.—Fungus saprophyticus; sporangiis sessilibus, hyalinis, spinosis vel hirsutis, sphaericis (7–50 μ), 2–5-papillatis; zoosporis sphaericis (2.5–3 μ), flagello 10–13 μ longo; sporis perdurantibus sphaericis (7–15 μ) vel ovalibus (5–6 $\times$ 7–14 μ), parietibus fuscis et verrucosis; prosporangiis germinantibus zoosporangia membranacea superficialia gerentibus.

Sporangia sessile, hyaline, predominantly spherical (7–50 μ) with 2–5 fairly prominent exit papillae, 3–4 μ by 4–6 μ broad; wall ornamented with short, simple bifurcate or dichotomously branched spines (2–6 μ high) or long (15–45 μ), simple or branched threads. Rhizoids fairly extensive (up to 120 μ) and richly branched; main axis up to 5 μ in diam. Zoospores spherical (2.5–3 μ) with a minute, spherical (0.3–0.5 μ), hyaline, refringent globule; flagellum 10–13 μ long. Resting spores spherical (7–15 μ) or oval (5–6 $\times$ 7–14 μ) with a thick (2–3.5 μ) brown, prominently-warted wall; content coarsely but evenly granular, growing out to form a superficial zoosporangium during germination.

Saprophytic on keratinized tissues, Porto Velho, Brazil; Dublin, Ireland; Groeningen, Holland; New Orleans, La.; Alden, Pawling and New York, N. Y.; Woods Hole, Mass.; Brookfield, Conn.; and Bergen County, N. J.

Although cat, dog, cow, camel and horse hair have been used as substrata, human hair has proven to be the best tissue for maintaining this species in culture. Blond or bleached hair is preferable for microscopic study because of its lack of pigment and relative transparency. The growth of the chytrid on this substratum is usually so prolific that the pieces of hair may be almost completely covered by sporangia and resting spores (fig. 1). The prolific production of resting spores, sporangia, and zoospores and the ease with which the resting spores may be induced to germinate make this species an ideal chytrid for laboratory use in mycology, although the rhizoids are not clearly visible in the substratum. The developmental stages of the sporangia and resting spores are shown in fig. 8 to 30, and since it is evident that they do not differ fundamentally from those of other *Rhizophydium* species, they will not be described at length. The present discussion will be limited, therefore, to the principal structural characters of the species.

Sporangia.—The outstanding structures of the sporangium are the spines and threads which arise from its outer wall. These begin as minute papillae on the incipient sporangium (fig. 10, 11). They may remain short (2–4 μ) and simple (fig. 7a) or branch dichotomously once to three times (fig. 7b, 7c, 14, 15, 16), or grow out into long (15–35 μ) simple or branched hairs (fig. 1, 2, 7d). The short bifurcate and dichotomously branched types are the most common and give the sporangia a marked prickled appearance in surface view. Regardless of the type present, they may be relatively few or very abundant, and no smooth-walled sporangia have yet been found. Sporangia with long hairs often have a hirsute appearance and resemble somewhat those of *R. chaetiferum* (Sparrow, 1937). As noted above in the diagnosis, the sporangia are predominantly spherical but they may be oval or slightly oblong also.

The wall of the sporangium is usually thin (1–1.5 μ), but in old and slowly maturing sporangia it may attain 3 μ in thickness (fig. 38). The exit

zoospores. $\times$1700.—Fig. 6. Zoospores swarming in partly empty sporangium. $\times$1400.—Fig. 7. Variations in shape and structure of spines and hairs on sporangia. $\times$1600.—Fig. 8–17. Developmental stages of sporangia and rhizoids. $\times$1600.—Fig. 18–21. Developmental stages of resting spores. $\times$1600.—Fig. 22, 23. Surface views of mature resting spores. $\times$1600.—Fig. 24–28. Germination stages of resting spores. $\times$1400.—Fig. 29. Zoospores from germinated resting spore. $\times$1800.—Fig. 30. Germinated resting spores with empty attached zoosporangium. $\times$1400.—Fig. 31, 32, 33. Surface views of basal plate with holes. $\times$2000.—Fig. 34. Cross section of basal plate showing constricted rhizoids growing through it. $\times$2000.—Fig. 35. Uninucleate zoosporangium. $\times$1400.—Fig. 36, 37. Tetra- and multinucleate zoosporangia with nuclei dividing. $\times$1400.—Fig. 38. Empty thick-walled zoosporangium. $\times$1400.—Fig. 39, 41, 42, 43. Uni-, bi-, tetra- and eight-nucleate resting spores. $\times$1700.—Fig. 40. Nucleus of uninucleate resting spore. $\times$2600.

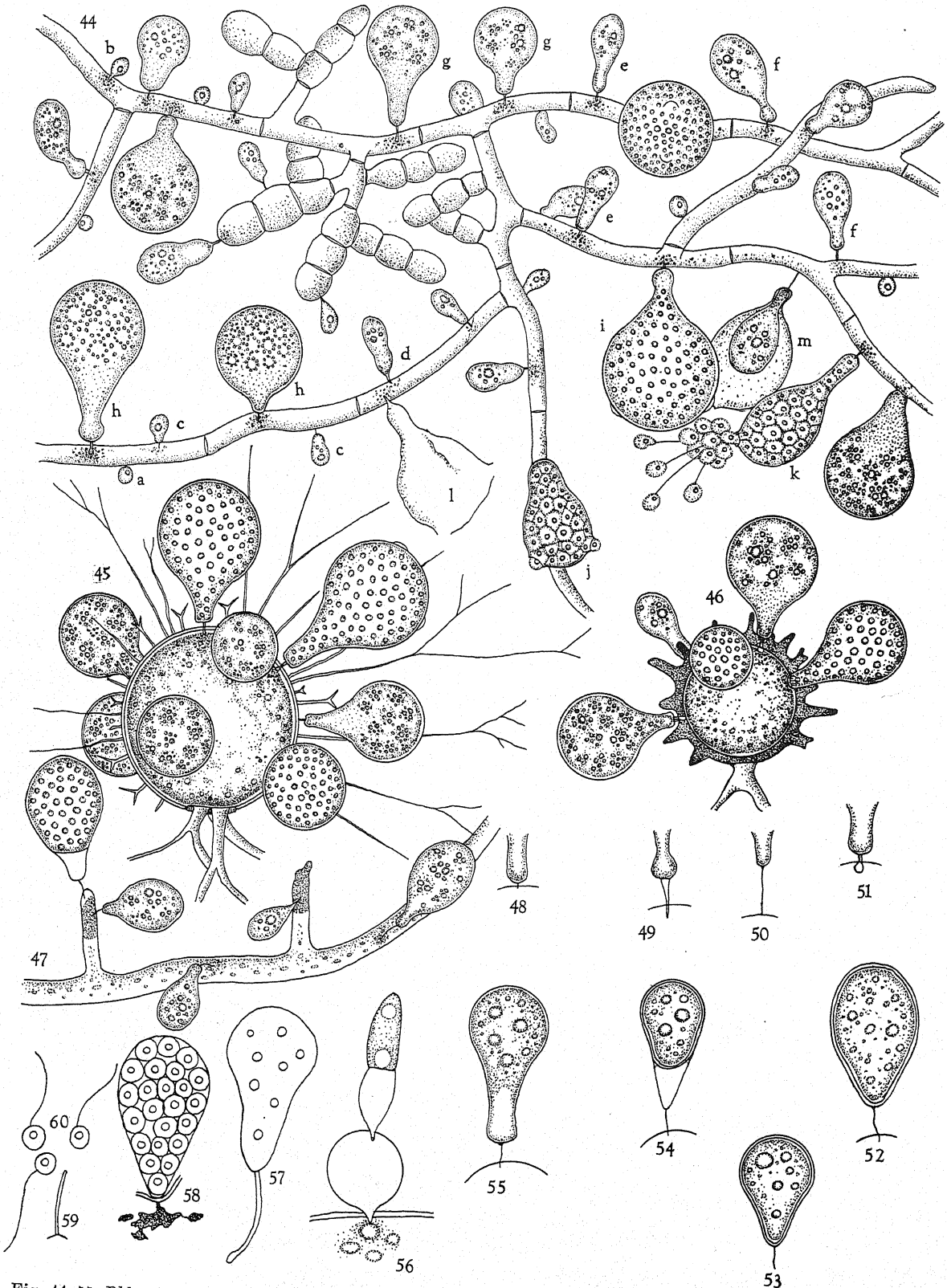


Fig. 44–55. *Phlyctidium mycetophagum.*—Fig. 56. *P. anomalum.*—Fig. 57–60. *Hapalopera piriformis.*—Fig. 44. Mycelium of a mucedinaceous fungus imperfectus parasitized by 37 thalli of *Phlyctidium mycetophagum* in various stages of development. ×1200.—Fig. 45. Sporangium of *Rhizophydium keratinophilum* infected with 8 thalli of

papillae are fairly broad and conspicuous (fig. 2, 17) and vary from two to five in number. The sporangia may be frequently torn loose from their rhizoids and the substratum in mounting the material for microscopic study (fig. 2, 3, 6), but this does not inhibit spore discharge if the sporangia are mature. Such sporangia are usually accompanied by several cells of the hair cuticle which are attached to a dense basal plate (fig. 2, 3, 6). In fixed, sectioned, and stained sporangia this plate is heavily stained and stands out sharply in contrast to the sporangial wall. As shown in fig. 31, 32, 33, and 34 it may be almost circular (4 to 11 μ in diam.), oval (4–11 $\times$ 5–14 μ), or slightly angular and irregular (4–8 $\times$ 6–14 μ) in outline and 1.8 to 3.5 μ in thickness. It is perforated by one to several circular or oval holes which correspond to the number of rhizoid attached to the base of the sporangium. As the rhizoids pass through these holes (fig. 32, 34, 38) they are usually constricted in those regions. The exact nature of this basal plate is not known. In fixed and stained preparations it appears to be a portion of the sporangium wall because of its similar staining reaction, but in living sporangia it seems to be composed of condensed cuticular cells which have become greatly modified by the presence of the fungus.

As the exit papillae deliquesce, the zoospores ooze out in dense streams (fig. 3) and remain inactive for a few seconds. Then, as their flagella uncoil they begin to shake and wiggle about and soon dart off. Those remaining in the sporangium swarm rapidly and emerge singly (fig. 6). They are spherical in shape while active and contain a minute refringent globule (fig. 4), but they frequently become intermittently amoeboid (fig. 5) and creep about. Within a short time they come to rest, lose their flagella, germinate, and develop into sporangia (fig. 8–17) or resting spores (fig. 18–23).

Rhizoids.—As noted earlier the rhizoids of *R. keratinophilum* are difficult to see in keratinized substrata, but by careful microdissection of the latter their structure and nature may be readily observed. They begin as branches of the germ tube (fig. 8, 9, 10, 11), and after penetrating the cuticle they spread out in the cortical layer of the hair and may also invade the medulla. They may arise from a large single axis at the base of the sporangium (fig. 14, 16) or from several axes (fig. 15, 18, 19), branch frequently, and extend for a distance of 120 μ along the length of the hair.

Resting spores.—The resting spores develop in the same manner as the sporangia (fig. 18–20) up to a certain stage, after which the wall thickens and turns light brown (fig. 21), while the content becomes coarse but evenly granular. The wall is covered with relatively short, conical and abruptly-tapering pegs or warts (fig. 22, 23). So far no evidence of sexuality has been found in relation to the formation of resting spores. However, most of the mature spores are binucleate (fig. 41), which suggests at least that they might be gametic in origin and dikaryotic. Occasional uni- (fig. 39), tetra- (fig. 42) and eight-nucleate (fig. 43) spores may occur also.

The resting spores germinate in one to two months under ordinary laboratory conditions, but germination may be accelerated by cold treatment of the spores in a refrigerator for a week. When brought back to room temperatures such spores germinate within a week or ten days. In germinating they function as prosporangia and give rise to small superficial zoosporangia, as shown in fig. 24 to 30. These secondary sporangia are of about the same diameter as the resting spores, spherical, oval or oblong in shape and with 1 to 3 exit papillae. Unlike the primary sporangia, they rarely bear spines (fig. 27) or hairs. The zoospores produced by them contain a slightly larger (0.8–1.2 μ) refractive globule (fig. 28, 29) than those formed in the primary sporangia, and in some spores two or three globules may be present.

PARASITE OF R. KERATINOPHILUM.—During our study of the material collected at Alden, N. Y., the sporangia and resting spores of *R. keratinophilum* became heavily infected with another chytrid which belongs to the genus *Phlyctidium*. Although it parasitizes a keratinophilic host, it is incapable of infecting and growing on keratinized substrata and cannot, therefore, be regarded as a keratinophilic chytrid. As shown in fig. 45 and 46, it may occur in great abundance on the sporangia and resting spores of *R. keratinophilum*, and in one case 22 parasites were found on a single host sporangium. However, this parasite is not obligate but may attack a large number of other fungi. Shortly after it was discovered on *R. keratinophilum*, tests were begun to determine whether or not it would parasitize other chytrids and larger fungi which were available at that time. So far it has been transferred to the following hosts: *Karlingia rosea, Rhizophydium coronum, Chytriomyces hyalinus, C. aureus, C. appendiculatus, Asterophlyctis sarcoptoides, Siphonaria variabilis, Septochytrium variabile, Polychytrium aggregatum, Rhizidiomyces hirsutus, Thraustotheca clavata, Aphanomyces* sp., *Achlya flagellata, Zoophagus insidians,* and a spe-

P. mycetophagum. ×1600.—Fig. 46. Infected resting spore of *R. keratinophilum.* ×1700.—Fig. 47. Mycelium and predaceous pegs of *Zoophagus insidians* infected with *P. mycetophagum.* ×1300.—Fig. 48, 49, 50, 51. Variations in length and structure of the rhizoid of *P. mycetophagum.* ×1800.—Fig. 52, 53, 54. Thick-walled bodies or resting spores(?) of *P. mycetophagum.* ×1600.—Fig. 55. Stage in the formation of a septate resting body. ×1600.—Fig. 56. Resting spore of *P. anomalum* (after Couch, 1932).—Fig. 57, 58. Sporangia of *Hapalopera piriformis.*—Fig. 59. Rhizoid or stalk after complete disappearance of sporangium wall.—Fig. 60. Zoospores of *H. piriformis* (after Fott, 1942).

cies of the family Mucedinaceae, Fungi Imperfecti. Of all fungi tested, the last-named species and *Karlingia rosea* were most heavily attacked, but no intensive checks were made to determine whether or not they are the most highly susceptible. Fig. 44 shows a portion of the mycelium of the fungus imperfectus with 37 sporangia of the parasite in various stages of development. As many as 120 parasites were found on a large sporangium and the rhizoids of *K. rosea,* and in this case the parasites were so closely packed together that their sporangia were angular in outline. Only the rhizoids of *R. coronum* were infected, and it appears as if the gelatinous sheath surrounding the sporangia of this host is an effective barrier to infection. In the case of *Z. insidians* the predaceous pegs as well as the rest of the mycelium were infected (fig. 47).

Filaments of *Cladophora glomerata, Spirogyra crassa, Vaucheria* sp., *Stigeoclonium* sp., *Oedogonium* sp., moss protonema, and pollen grains of various gymnosperms and angiosperms were tested also, but they did not become infected. The parasite, accordingly, seems to be limited in host range to fungi.

Inasmuch as the parasite is relatively small a single infection does not cause any marked visible changes in the host cell, particularly if the latter is very large as in some sporangia of *K. rosea.* In the case of the chytrid host sporangia, they fail to develop and mature if infected in the early developmental stages. The first visible effect of infection is a clumping and increase in refractive index of the host protoplasm around the rhizoid or haustorium of the parasite, as shown in fig. 44. As the parasite develops a considerable portion of the protoplasm is absorbed, especially in small host cells, while the remainder degenerates.

This parasite is characterized by smooth, hyaline, predominantly obpyriform sporangia with a tapering base and 1 to 4 low, inconspicuous, apical or subapical exit papillae, minute spherical zoospores, and by an unbranched basal rhizoid which is usually fine and filamentous, or rarely knobbed or peg-like. Because of the nature of its rhizoid, it belongs in *Phlyctidium* on the basis of present-day interpretations of this chytrid genus. The generic dictinctions of *Phlyctidium* are not sharply defined, and although it is not regarded as a valid genus by all chytridiologists, they continue to recognize it for practical purposes. It includes at present approximately twelve species, several of which are so incompletely known that they are of doubtful validity. Of these twelve species, only two have been reported to parasitize fungi: *P. anomalum* on *Phlyctidium bumilleriae* Couch (1932) and *P. dangeardi* serbinow (1907) on prosporangia of *Saccomyces.* The latter species is very doubtful inasmuch as no rhizoids have been observed. *Phlyctidium anomalum,* on the other hand, has been transferred to a new genus by Whiffen (1942) and named *Septosperma anomala,* leaving *R. dangeardi* as the only species parasitic on fungi.

As far as shape of sporangia and the appearance of the unbranched rhizoid are concerned, the species found by the author on *R. keratinophilum* resembles *Hapalopera piriformis* Fott (1942), whose sporangia and zoospores are shown in fig. 57 to 60 for comparison. However, the latter species parasitizes an alga, *Characium ancora,* and its sporangium wall disappears completely during dehiscence as in *Solutoparies Pythii* Whiffen (1942), leaving only the stalk or rhizoid (fig. 59). These two characteristics distinguish it from our parasite, which we regard as a new species and name *Phlyctidium mycetophagum* because it attacks the sporangia, resting spores, rhizoids and mycelium of various fungi.

Phlyctidium mycetophagum n. sp.—Fungus parasiticus; sporangiis hyalinis, laevibus, obpyriformibus (8–30 × 10–25 μ), rare sphaericis aut ovalibus; 1–4-papillatis; zoosporis sphaericis (2–2.9 μ); flagello 8–10 μ longo; rhizoidiis tenuibus (2–8 × 0.8–1.2 μ), eramosis; sporis perdurantibus dubiis.

Sporangia hyaline, smooth predominantly obpyriform (8–30 μ high by 10–25 μ in greatest diameter) with a tapering or slightly inflated base, occasionally almost spherical or oval, with 1–4 low, inconspicuous, apical or subapical exit papillae. Zoospores spherical (2–2.9 μ) with a minute (0.5–0.8 μ) refringent, hyaline globule; flagellum 8–10 μ long. Rhizoid unbranched, usually thread-like or filamentous, up to 8 μ long and 0.8–1.2 μ in diameter, rarely knobbed at the end, or peg-like. Resting spores doubtful.

Parasitic on the sporangia, resting spores, rhizoids and mycelium of *Rhizophydium keratinophilum, R. coronum, Karlingia rosea, Chytriomyces hyalinus, C. aureus, C. appendiculatus, Siphonaria variabilis, Asterophlyctis sarcoptoides, Septochytrium variabile, Polychytrium aggregatum, Rhizidiomyces hirsutus, Thraustotheca clavata, Aphanomyces* sp., *Achlya flagellata, Zoophagus insidians,* and a species of Fungi Imperfecti, Alden, N. Y., and New York City.

The developmental stages of this species are shown in fig. 44a to 44m. As in other species of *Phlyctidium* the zoospore comes to rest on the host cells (fig. 44a) and develops a short, fine germ tube (fig. 44b) which with maturity becomes drawn out into a filament (fig. 44c) or may become slightly knobbed (fig. 44f, h, k) or peg-like (fig. 44d, g, i), while the zoospore body enlarges to become the zoosporangium (fig. 44h, i, k). The internal protoplasmic changes during the development and maturation of the sporangia are fundamentally similar to those reported for other eucarpic, rhizidiaceous chytrids and do not need detailed description. Occasionally, the sporangia may become septate, as shown in fig. 47a. In this process, the protoplasm in the tapering base becomes highly vacuo-

late and sparse and migrates up toward the expanded portion of the sporangium, after which a septum is formed. Occasionally, the base may be slightly inflated (fig. 44f, h, i).

The length of the rhizoid within and outside of the host cell varies considerably. It may be fairly long (fig. 50) so that the sporangia appear to be attached to the host cell by a fine filament, or very short (fig. 48). In the latter cases, the sporangium may appear to be sessile. Also in rare instances the end of the rhizoid may be slightly inflated or knobbed (fig. 51), or the whole rhizoid may taper and become peg-like (fig. 49). The depth to which it penetrates the host cell is often difficult to determine because of the density of the host protoplasm clustered around it. That the rhizoid extends into the host cell has been clearly observed a number of times. However, in some cases, it seems to be merely attached to the outer surface of the host wall (fig. 50) and in others to extend into the layers of the wall. The clumping of the host protoplasm around the points of infection in such cases, however, indicate that the rhizoid may have entered the cell. The rhizoid is tough but fairly flexible, so that the sporangia may be moved back and forth without breaking off. It may also be pulled out of the host cell with the attached sporangium. Detached sporangia, if mature, may readily undergo cleavage in water and discharge their spores normally.

In addition to the thin-walled sporangia shown in fig. 44, 45, and 46, a few thicker-walled bodies have been found which may be the resting spores, although they look more like the slowly-maturing or dormant sporangia that occasionally occur in chytrid species. These bodies are usually oval (7–10 × 9–15 μ) or pyriform (6–12 × 8–18 μ), smaller than the ordinary sporangia and thicker-walled (fig. 52–54). Occasionally, they are two-celled (fig. 54) and somewhat similar to the resting spores of *Septosperma anomala* (fig. 56). Such spores apparently develop as a result of the concentration of the protoplasm in the upper portion (fig. 55) after which septation and thickening of the wall occur. Germination of these bodies has not been observed so that it is not certain whether they are true resting spores or merely dormant, thick-walled sporangia. Resting spores apparently occur only rarely in *Phlyctidium*, and so far they have been found in only four of the dozen or more described species. Therefore, it is not possible to make extensive comparisons with the thick-walled bodies found in *P. mycetophagum*.

SUMMARY

Keratinized animal tissues like skin, hair, finger and toe nails, hoofs, horns and feathers are excellent substrata as bait for isolating and growing certain specialized chytrids. Since such species appear to be limited in occurrence and growth to keratinized tissues and apparently require *keratin* as a nutrient, they are described as keratinophilic chytrids.

Rhizophydium keratinophilum n. sp., the first of these species to be described, was isolated first from soil and water from an abandoned and heavily eroded cemetery near Porto Velho, Amazonas, Brazil. Later, it was found in soil from Ireland, Holland and several of the northeastern and southern states of the U. S. A. So far it has been grown successfully on most keratinized tissues except human skin, but all attempts to grow it on agar media containing cystine and hydrolysates of hair and pig skin have failed. This species is characterized by hyaline, predominantly spherical sporangia which are ornamented by short, simple, bifurcate, or dichotomously branched spines, or simple or branched hairs, spherical zoospores, and by light-brown, warty resting spores.

Sporangia and resting spores of this species collected at Alden and in New York City, N. Y., became heavily infected by another chytrid, *Phlyctidium mycetophagum* n. sp., which may parasitize other chytrids and larger fungi also. This parasite is characterized by predominantly obpyriform, tapering sporangia and a filamentous, rarely knobbed or peg-like, unbranched rhizoid.

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THE INHERITANCE OF ASCORBIC ACID CONTENT IN CABBAGE [1]

J. C. Walker and R. E. Foster[2]

In a recent paper, Smith and Walker (1946) emphasized the influence of heredity on the ascorbic acid (vitamin C) content of cabbage. Their studies revealed that while environmental factors such as season of growth, time of harvest, and possibly rainfall and amount of sunlight may affect the total amount of this vitamin in cabbage heads, significant differences occur between cabbage varieties and between certain strains or lines within a variety. They found that among the varieties tested, All Head Select usually contained the least amount of the vitamin while Jersey Queen contained the most. A line of Wisconsin All Seasons, derived by a number of generations of close breeding, was discovered to have an unusually high ascorbic acid content. During four consecutive years, repeated analyses of heads from progenies from selfed or sib-crossed individuals showed that the high-vitamin content was relatively stable within this line. F_1 hybrids between individual plants from this strain and from low-vitamin strains were reported to have an ascorbic acid content intermediate between the two parents.

The purposes of the present work were: (1) to make use of the high-vitamin line of cabbage as parental material from which, by hybridization and selection, the factors for high ascorbic acid content could be incorporated into standard commercial varieties; (2) to study the mode of inheritance of factors influencing high production of this vitamin in cabbage. In this paper are included the F_1 data from twenty-three crosses and the F_2 data from eight of those crosses. Inasmuch as it was not possible to secure complete data from the F_3 generation, which admittedly would add much to the genetic interpretation of the inheritance of ascorbic acid content, only the F_1 and F_2 data are presented in this report.

Methods and materials.—The three-slice method of analysis of cabbage heads described by Smith and Walker (1946) was used without modification to determine the ascorbic acid content of all cabbage studied. In 1944, the recommended six-head sample was used to obtain the content of the parent and F_1 lines. Since segregating F_2 populations were studied in 1945, all available heads were analyzed to determine frequency distributions in the parent lines as well as in the F_1 and F_2 progenies. With the hope of facilitating future seed production by preserving cabbage heads on the plants, a limited attempt was made to correlate ascorbic acid content in the outer leaves with that in the head. Since poor correlation seemed to exist and the analyses of outer leaf tissue were found not reliable enough to warrant use, the method was abandoned.

Crosses between individuals from the high-vitamin line and low-vitamin individuals from several varieties were made in the greenhouse during the winter of 1943–44. The parent plants were also selfed or sib-crossed. It should be pointed out here that the more desirable practice of repeated selfing to stabilize characters cannot always be followed in cabbage because of the rather rapid loss of vigor which results. It could not be expected, then, that all high-vitamin parents used would be homozygous for all factors influencing ascorbic acid content. In 1944, seedlings of the parents and of the hybrids grown in outdoor seed beds were selected at random. The earlier varieties were set out in 50-plant rows in the field on June 29; the later varieties were transplanted on July 10. Heads for the vitamin determinations were harvested at random as they became mature.

F_1 plants, chosen at random, were sib-crossed in the greenhouse during the winter of 1944–45 to obtain F_2 seed. This seed and seed of all parental material available was sown in an outdoor seedbed on May 8, 1945, and seedlings were transplanted into the field on July 2. F_2 progenies were planted in 100-plant rows; parent and F_1 lines were in 50-plant rows. All available heads were harvested for analysis. An individual record was kept of each plant in the F_2 population so that certain headless stumps of plants desirable with respect to ascorbic acid content and horticultural type might be saved for future seed production.

Lots 25–484 through 25–497, originating from the high-vitamin line, were used as the high-vitamin parents. Low-vitamin parents were chosen for characters, other than ascorbic acid content, that made them particularly desirable. Three standard yellows-resistant varieties were used, which may be identified in the progenies by their respective key numbers as follows: 25–, Wisconsin All Seasons; 83–, Improved Wisconsin Ballhead; 40–, All Head Select. The high-vitamin line was derived from Wisconsin All Seasons. Certain low-vitamin parents were also chosen from F_2 populations from crosses between certain other standard yellows-resistant varieties which may be identified as follows: 1922–, Resistant Detroit × Wisconsin Golden Acre; 2221–, Wisconsin Golden Acre × Racine Market; 2135–, Racine Market × Globe.

Experimental results.—*F_1 progenies.*—In table 1 are presented the data from F_1 progenies of twenty-three crosses between high- and low-vitamin parents as well as from self or sib progenies from both parent plants. Comparison is made between

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TABLE 1. *Ascorbic acid content of parent lines and F_1 hybrids expressed in mg. per 100 g. fresh tissue.*

Cross	Low-vitamin parent	F_1	High-vitamin parent	Means of parents		Deviation of F_1 from mean of parents	
				Arith.	Geom.	Arith.	Geom.
25–310 × 25–494	52.5	65.1	91.7	72.1	69.4	—7.0	—4.3
25–312 × 25–497	47.5	66.6	82.7	65.1	62.7	+1.5	+3.9
25–315 × 25–496	49.8	73.9	83.5	66.7	64.5	+7.2	+9.4
25–323 × 25–486	54.4	78.1	89.9	72.2	69.9	+5.9	+8.2
25–328 × 25–484	44.1	68.0	80.5	62.3	59.6	+5.7	+8.4
25–334 × 25–495	69.0	70.1	90.5	79.7	78.9	—9.6	—8.8
25–336 × 25–494	58.1	72.3	91.7	74.9	73.0	—2.6	—0.7
25–340 × 25–497	57.3	73.0	82.7	70.0	68.8	+3.0	+4.2
25–413 × 25–495	58.0	69.8	90.3	74.2	72.4	—4.4	—2.6
25–428 × 25–495	53.3	65.0	90.3	71.8	69.4	—6.8	—4.4
25–454 × 25–492	69.1	79.0	91.5	80.3	79.5	—1.3	—0.5
25–460 × 25–493	62.1	80.0	83.1	72.6	71.8	+7.4	+8.2
25–473 × 25–496	64.0	71.0	83.5	73.8	73.1	—2.8	—2.1
83–751 × 25–496	45.1	55.0	83.5	64.3	61.4	—9.3	—6.4
83–754 × 25–492	38.7	56.9	91.5	65.1	59.5	—8.2	—2.6
83–766 × 25–493	44.3	50.9	83.1	63.7	60.7	—12.8	—9.8
83–835 × 25–494	44.4	66.0	91.7	68.0	63.7	—2.0	+2.3
83–838 × 25–495	45.6	64.6	90.5	68.0	64.2	—3.4	+0.4
83–846 × 25–496	46.8	59.4	83.5	65.2	62.5	—5.8	—3.1
83–856 × 25–496	59.7	66.2	83.5	71.6	70.6	—5.4	—4.4
40–44 × 25–484	60.9	70.4	80.5	70.7	70.0	—0.3	+0.4
2135–0–15 × 25–489	43.7	58.9	84.0	63.9	60.6	—5.0	—1.7
2221–0–11 × 25–486	45.8	59.1	89.9	67.9	64.2	—8.8	—5.1
Means	52.8	66.9	86.7	69.7	67.4	—2.8	—0.5

the mean ascorbic acid content of six or more plants from each parent line and that from the corresponding F_1 with the arithmetic and geometric means of the two parents. In most cases the mean of the F_1 hybrid approximated both the arithmetic and the geometric mean of the parents; in fifteen cases the F_1 mean was closer to the geometric mean than to the arithmetic mean; in eight cases the opposite was true. In fourteen cases the F_1 mean was less than either the arithmetic or the geometric mean of the parents; in six cases it was more; and in two cases it fell between these means. The intermediate position of the ascorbic acid content in the F_1 progenies suggests that the character was inherited as a quantitative one. In one progeny (25–334 × 25–495) the F_1 mean was very close to that of the low-vitamin parent which suggests that in this case the genes governing ascorbic acid content were few in number and that those for low ascorbic acid content were dominant.

The work of Smith and Walker (1946) showed that variability in ascorbic acid content within a given stains, as secured at Madison, was associated most directly with different dates of transplanting and corresponding maturity. In the present studies, the F_1 and parental progenies derived from crosses within the variety Wisconsin All Seasons (25–) and from the crosses between that variety and Improved Wisconsin Ballhead (83–) were all planted on the same day and they matured at approximately the same time. It is believed that under these circumstances the intermediate position of the F_1 means was due primarily to genetic factors rather than to environmental influences.

F_2 progenies.—Analyses were secured from all heads available in the F_2 progenies grown in 1945. When the parent lines and F_1 progenies were grown, heads from them were analyzed also. In table 2 are given the frequency distributions of F_2 progenies together with available P_1 and F_1 progeny data. Although the populations analyzed were often smaller than desirable for an exact inheritance study, the data are of considerable interest in indicating the type of inheritance involved. Where All Head Select, Wisconsin Hollander, and Wisconsin All Seasons were used as low-vitamin parents, the parent lines, the F_1 progenies and the F_2 progenies matured at approximately the same time. It is believed, therefore, that the environmental factors which have been shown to have some effect upon ascorbic acid content (Smith and Walker, 1946) had minor influence on the variation between lines and individuals used in these comparisons.

It is to be seen that, in general, the frequency distribution of the individuals in the F_2 progenies approximated a normal curve. The range of ascorbic acid content was wider for the F_2 than for either parent, as is shown by the standard deviations. The F_2 progenies varied, however, in the difference between their standard deviations and those of the respective parent progenies. It is evident that the low-vitamin plants selected as par-

TABLE 2. *Frequency distribution of ascorbic acid content in high- and low-vitamin parents and in F_1 and F_2 hybrid progenies.*

Hybrid combination (family)	Progeny number	Generation	Class centers in milligrams per 100 grams fresh tissue												Number of heads	Arith. mean	Standard deviation
			37.5	42.5	47.5	52.5	57.5	62.5	67.5	72.5	77.5	82.5	87.5	92.5			
A	40–44*s*	P_1	..	..	8	11	9	1	1	..	..	..	..	..	30	53.6	4.84
	25–484	P_1	..	..	..	..	..	..	..	3	9	5	3	1	21	80.1	5.39
	40–44 × 25–484	F_1	..	..	..	2	3	6	4	..	..	..	..	..	15	60.8	5.07
	40–44 × 25–484	F_2	3	1	3	9	16	16	16	13	5	2	1	..	85	65.0	10.05
B	*83–856s*	P_1	..	..	6	9	12	15	8	3	..	..	..	..	53	59.3	6.94
	25–496	P_1	..	..	..	..	..	1	4	5	2	1	..	..	13	71.3	5.34
	83–856 × 25–496	F_2	..	3	7	13	16	10	28	11	10	3	1	..	102	63.8	10.06
C	*83–751s*	P_1	..	14	23	10	3	..	..	..	..	..	..	..	50	47.4	4.24
	25–496	P_1	..	..	..	..	..	1	4	5	2	1	..	..	13	71.3	5.34
	83–751 × 25–496	F_2	..	..	14	28	20	26	8	2	..	..	..	..	98	57.4	6.41
D	*25–496*	P_1	..	..	..	..	..	1	4	5	2	1	..	..	13	71.3	5.34
	25–315 × 25–496	F_2	..	..	..	3	9	13	29	23	12	6	2	..	97	69.0	7.60
E	*25–486*	P_1	..	..	..	..	..	1	2	11	11	5	2	..	32	75.9	5.57
	2221–0–11 × 25–486	F_2	..	..	2	9	30	26	12	5	1	1	..	..	86	61.0	6.27
F	*25–323*	P_1	..	1	11	25	10	4	..	..	..	..	..	..	51	52.9	4.50
	25–486	P_1	..	..	..	..	..	1	2	11	11	5	2	..	32	75.9	5.57
	25–323 × 25–486	F_2	..	..	..	4	10	30	27	12	7	3	..	..	93	66.1	6.70
G	*25–334*	P_1	..	..	..	2	12	26	6	2	..	..	..	..	48	61.6	4.21
	25–334 × 25–495	F_1	..	..	..	..	4	8	16	12	2	3	..	..	45	68.5	5.90
	25–334 × 25–495	F_2	..	..	1	4	19	25	26	15	2	..	..	..	92	64.0	6.22
H	*25–336*	P_1	..	..	..	6	20	15	10	4	2	1	..	..	58	62.4	6.84
	25–336 × 25–494	F_2	..	..	3	6	11	23	16	21	14	3	4	1	102	67.7	9.50

ents differed in the genes governing ascorbic acid content. For instance, 83–751 and 83–856 represented two inbred lines from the same variety. The selfed progenies of each plant showed that in 1945 the mean of 83–751 was 11.9 mg. lower than that of 83–856. These were crossed with the same high-vitamin plant (25–496), and in 1944 the means of the F_1 progenies were intermediate in ascorbic acid content (table 1), but in each case it was less than the arithmetic and the geometric means of the respective parents. In the case of 83–856 × 25–496 the standard deviation of the F_2 was nearly twice that of either parent; in the case of 83–751 × 25–496 it was 20.0 per cent and 51.0 per cent greater than the respective parents. In the case of the F_2 with the larger standard deviation the full range of parental types was recovered; in the other case the two highest classes of the high parent and the lowest class of the low parent were not recovered. The same high-vitamin parent (25–496) was crossed with another parent (25–315) which showed a very low vitamin content in 1944 (table 1). In this case the standard deviation of the F_2 was 42.3 per cent greater than that of the high-vitamin parent, and all of the ascorbic acid classes of that parent were recovered.

In four cases the F_2 was compared directly with each parent of the respective cross. In one family (family B, table 2), certain individuals were recovered in the F_2 that were in one class higher as well as some in one class lower than those of either parent. In one case (family A, table 2) individuals were recovered in the F_2 in two classes lower than the lowest class represented in the low-vitamin parent and in all but the highest class represented in the high-vitamin parent. In one case (family C, table 2) the lowest class of the low parent and the two highest classes of the high parent were not recovered; in the fourth case (family F, table 2) the two lowest classes of the low parent and the highest class of the high parent were not recovered. In five out of six F_2 progenies, the high-vitamin parent progenies of which were tested, individuals were found which were as high or higher than those in the next to highest class of the high-vitamin parent.

Discussion.—The primary purpose of this study was to determine what type of inheritance could be expected when an inbred line of cabbage high in ascorbic acid content was used as a parent in hybrid combinations with standard yellows-resistant varieties which showed a much lower range of acid content. It is evident from the data presented that ascorbic acid content is inherited as a quantitative character and that the level of ascorbic acid attainable in a given line or individual is determined by the combination of genes present, but the expression of the character may be influenced by environmental factors. While in some of the F_2 progenies the range of ascorbic acid content was wider than that of both parents the limited data do not warrant interpretation of this phenomenon as one of the transgressive segregation.

The selection of varieties for disease resistance and various horticultural characters without reference to ascorbic acid content may lead to strains with a level of ascorbic acid which is low regardless of environment. An example of this is found in All Head Select, a yellows-resistant variety represented in this study by the parent plant 40–44. In the crosses between an inbred line high in ascorbic acid content selected from Wisconsin All Seasons and low-vitamin individuals from this and three other varieties, the extraction of high ascorbic acid classes in the F_2 progenies indicates that average ascorbic acid content of these and presumably other varieties may be raised by a process of controlled crossing and selection.

SUMMARY

In twenty-three F_1 hybrid progenies from crosses between an inbred line of cabbage high in ascorbic acid content and standard yellows-resistant varieties which had a much lower range of acid content, the ascorbic acid mean value was intermediate between mean values of the corresponding parent progenies. In all but one case the mean of the F_1 was not far removed from the arithmetic and geometric means of the parents.

In eight F_2 progenies studied the frequency distribution of the ascorbic acid values of individual heads approximated a normal curve. The range of ascorbic acid content was wider for the F_2 than for parent progenies, when these were tested comparatively. The F_2 progenies varied in the difference between their standard deviations and those of the respective parent progenies, indicating that the parent plants selected differed in the genes governing ascorbic acid content.

It is concluded that ascorbic acid content is inherited as a quantitative character and that the level of acid content attainable in a given line or individual is determined by the combination of genes present which govern this character, while the expression of the character may be influenced to some extent by environmental factors.

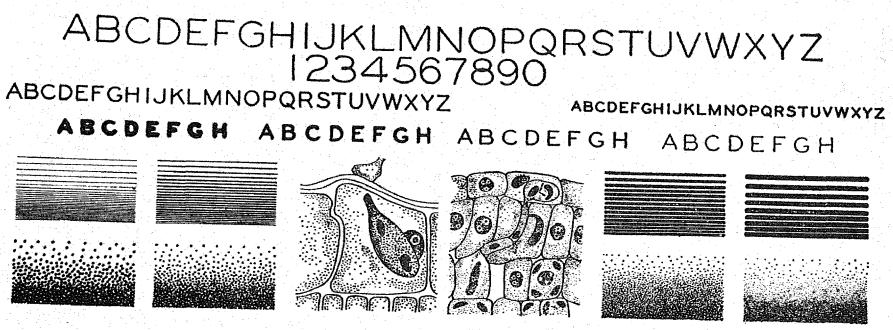
Studies of crosses between the high ascorbic acid inbred and standard yellows-resistant varieties indicate that the average ascorbic acid values of these and presumably other varieties may be raised considerably with retention of the major horticultural characteristics of the variety concerned by a process of controlled crossing and selection.

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LITERATURE CITED

Smith, F. G., and J. C. Walker. 1946. Relation of environmental and hereditary factors to ascorbic acid in cabbage. Amer. Jour. Bot. 33: 120–129.





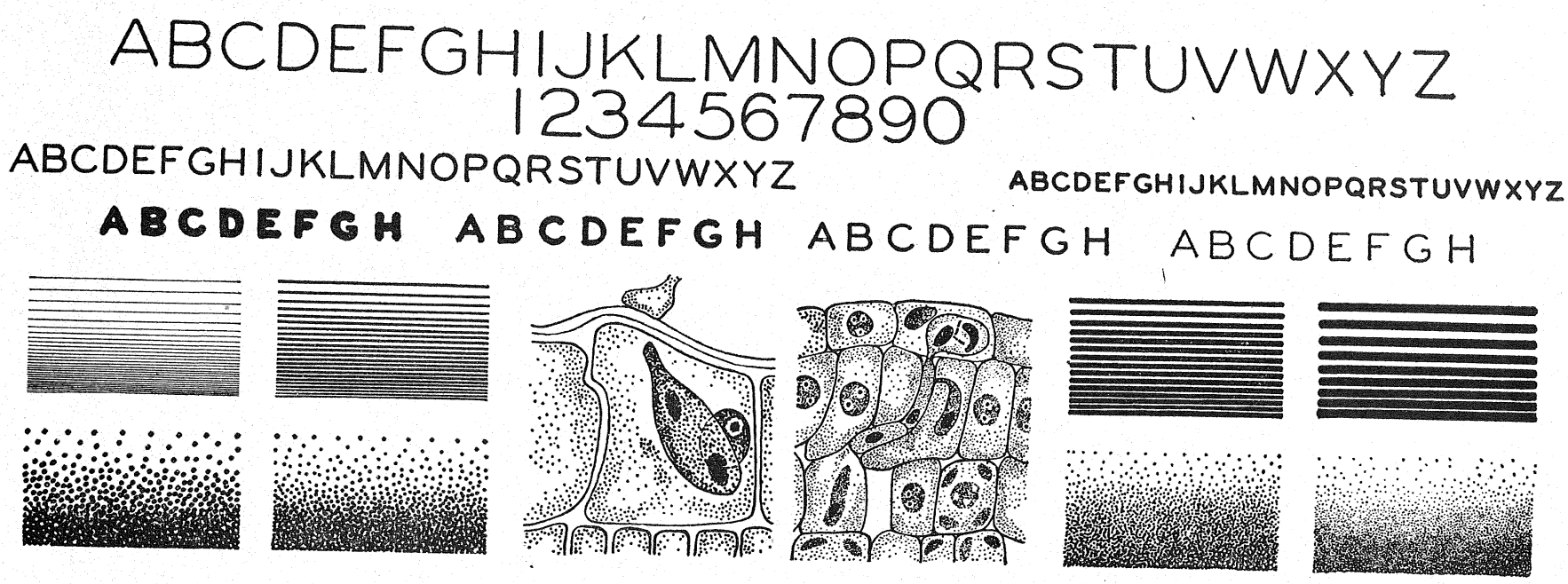
DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to ¼. Middle—Reduction to ½. Bottom—Original size.

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Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.



SOME FACTORS AFFECTING FRUIT PRODUCTION BY CRYPTOSTEGIA [1]

J. T. Curtis

One of the methods used in obtaining rubber from plants of the genus *Cryptostegia* R. Br. consists of the collection of latex from unripe fruits (Curtis and Blondeau, 1946). Because of the possible importance of this source of rubber in the recent emergency, a study was made of some of the factors affecting fruit set and fruit production. The present paper reports those phases of the work which appear to be of interest from the standpoint of general reproduction physiology.

Materials and methods.—The investigations were conducted at an experimental plantation near Gonaives, Haiti, and at a series of subplots in various soil and climatic regions of Haiti (Symontowne, 1943). Both species of *Cryptostegia, C. grandiflora* (Roxb.) R. Br. and *C. madagascariensis* Bojer, as well as the interspecific hybrid were used in the study. Plant material of *C. grandiflora* was derived mostly from seeds collected in Haiti, although seedlings originating in India, Australia, Mexico and other islands of the West Indies were also employed. The *C. madagascariensis* plants were from two sources—Port-au-Prince, Haiti, and the U. S. Plant Introduction Gardens at Coconut Grove, Florida. The F_1 hybrid clones were propagated from plants growing at Thorland, near Port-au-Prince, Haiti.

Certain characteristics of the inflorescence and flower of *Cryptostegia* are of importance for an understanding of fruit set; these will be described briefly. The large and showy flowers (fig. 1) are borne at the branch tips in dichotomous cymes. The terminal flower is the first to open. It is followed by a pair of flowers, each on a separate branch originating below the first flower. In *C. grandiflora,* the branches continue to bear single flowers, as in a cincinnus, with the flowers on the two branches opening in pairs. Ordinarily, the pairs are separated by 6 days in anthesis. The average number of pairs is seven, thus giving an average total of 15 flowers per inflorescence. In *C. madagascariensis,* the trichotomous branching is repeated two to four times, with resulting mature inflorescences containing up to a maximum of 16 branches. The average number of sets of flowers is 10, with a total of 36 flowers per inflorescence. The branches in both species are conspicuously jointed between successive flowers; they possess abscission layers at each joint.

The flowers of the two species differ chiefly in the position of the glands within the corolla tube. Those of *C. grandiflora* converge at the tips, allowing entrance to the lower flower parts only between the bulbous bases of the glands (fig. 2), while those in *C. madagascariensis* are separate, permitting direct entrance to stigma and style (fig. 3). In both species, the flowers remain open 24 hours or less, with the majority falling after 14 hours, regardless of whether they are pollinated or not. This abscission results in the dropping of only corolla, stamens, stigma and style. The ovaries, calyx and receptacle remain attached for an additional 80 hours in non-pollinated flowers.

A description of the interspecific hybrid, accompanied by excellent illustrations, was given by Polhamus, Hill, and Elder (1934).

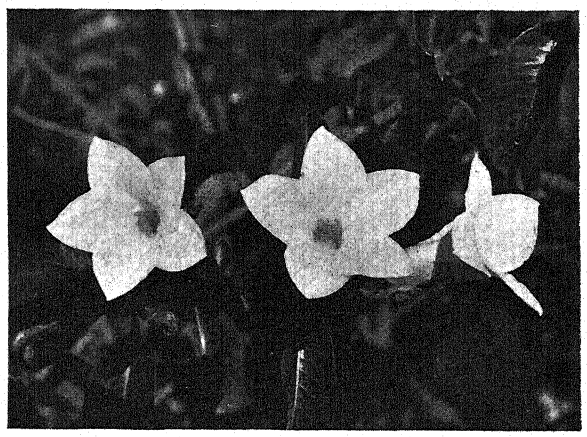


Fig. 1. Flowers of *Cryptostegia grandiflora.*

The studies here reported were conducted on cultivated plants grown in plantation fields from transplanted seedlings. Several spacings were used, varying from 540 to 14,300 plants per acre. Most observations were made on plants at a density of 3000 per acre, planted in single rows 8 feet apart, with the plants 1.5 feet apart in the rows. Much of the experimental material consisted of clones grafted on *C. grandiflora* stocks. The grafts were of the side insert cleft type, made at or near the crown level of the stocks.

Flower production was determined by counting all open flowers on individual plants, on uniform rows or blocks of plants, and on all individuals of several clonal lines. The counts were made daily at 11 a.m., when maximum numbers of flowers were present, and were continued for 790 consecutive days from January, 1943 to March, 1945. Fruit production on the same plants was determined at monthly intervals by counting all fruits that had set in a 30 day period.

Experimental results.—*Seasonal variations.*—Flowers are produced by both species of *Cryptostegia* at all times of the year but the number varies greatly with season, being highest in the summer.

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The average number of flowers per plant per week for field-grown plants of *C. grandiflora* for the 2 year period February, 1943 to January, 1945, is shown in fig. 4. The same figure also shows the number of fruits per plant per month and the total rainfall per month. The plants were transplanted seedlings 4 months old at the start of the experiment; they had been in bloom for only 1 month. These particular plants were never pruned or interfered with in any way, since they were employed as controls in contemporary rubber research (Sy-

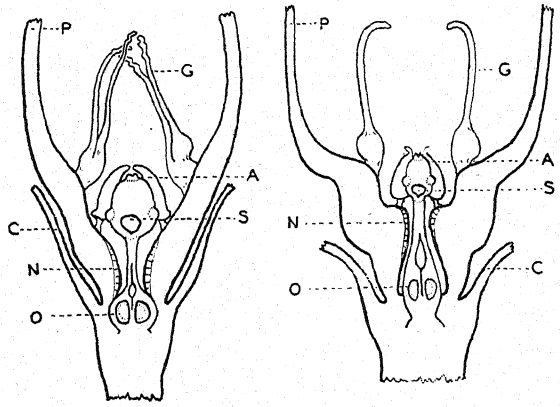


Fig. 2-3.—Fig. 2 (left). Diagrammatic sketch of longitudinal section of central portion of *C. grandiflora* flower, showing relation of corolla glands to anthers, stigma and nectary. Translators are not shown. P, petal; G, corolla gland; A, anther; S, receptive surface of stigma; C, calyx; N, nectary; O, ovary.—Fig. 3 (right). Diagrammatic sketch of longitudinal section of central portion of *C. madagascariensis* flower, showing relation of corolla glands to anthers, stigma and nectary. Translators are not shown. P, petal; G, corolla gland; A, anther; S, receptive surface of stigma; C, calyx; N, nectary; O, ovary.

montowne, 1943). The average height of their crowns increased from 3 feet at the start of the experiment to 8 feet at the end, and the basal diameter of their trunks increased from 18 mm. to 92 mm.

The lack of coincidence between the periods of maximum flowering and maximum fruit set was demonstrated even more clearly in the case of *C. madagascariensis*, as shown in fig. 5, where monthly flower and fruit production for 1944 is presented. Flowers occurred in greatest numbers in July, while fruiting was confined to a relatively narrow season with a peak in October. Both species agreed in showing the greatest reproductive efficiency (total number of fruits per plant expressed as a percentage of the total number of flowers present in the same period) in November, with low points in July (fig. 6).

Flower production of both species was highest when conditions for vegetative growth were optimum. The long days and relatively high rainfall of the summer season combined to give maximum growth which resulted in many branches of the flowering type. Total weight of vegetative material as determined by periodic harvesting of plant tops showed a seasonal response paralleling that of flower production; rubber production by the whip-tapping method reacted in a similar way (Blondeau and Curtis, 1946).

Certain other plants show a similar lack of temporal correlation between flowering and fruiting. Went and Cosper (1945) reported a considerable lag period between the production of flowers and the first fruit set in tomato, the time varying from 14 to 57 days in different seasons. They interpreted this as due to different night temperature requirements for flowers and fruits, with the fruits dependent upon lower temperatures. An effort was made to find some explanation for the observed periodicities in *Cryptostegia* by means of a careful comparison between fruit set and all of the measured environmental factors. No positive correlation with day or night temperature, relative humidity or wind velocity could be found. Similarly, no direct relation was apparent between day length and fruit production. In *C. grandiflora*, the best correlation was with rainfall, as the fruiting maxima occurred two months after each rainfall peak (fig. 4). Flower production, however, was independent of rainfall, since the main flowering peaks were recorded in August of each year, with secondary maxima in November, although the distribution of rainfall was very different in the two years.

Fruit set in *C. madagascariensis* was not directly related to either temperature or rainfall. The only positive correlation was with the season of high night humidity, as indicated by the number of nights per month in which the dewpoint was reached. This environmental measure attained a peak of 21 nights in October and was only 5 nights or less for the

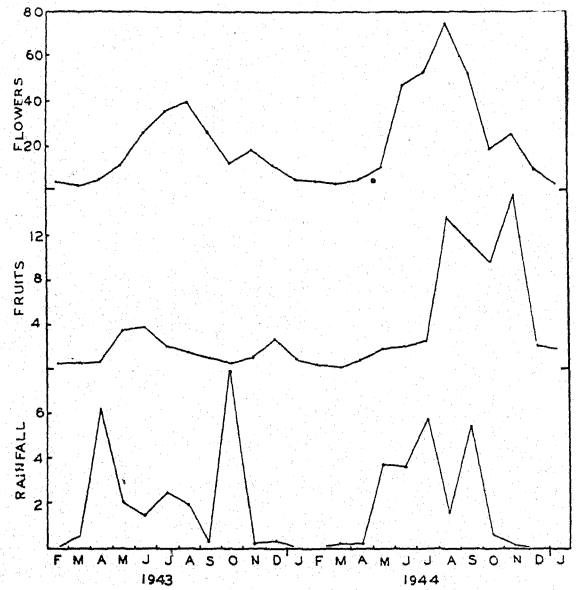


Fig. 4. Average number of *C. grandiflora* flowers per plant per week by months, average number of fruits set per plant per month, and rainfall in inches per month at Gonaives, Haiti, for the period from February, 1943 to January, 1945.

months from January through July. Carlson (1932) reported that the rate of fruit set in alfalfa also was correlated with atmospheric humidity, although optimum conditions for this plant were at medium relative humidity values (36 per cent to 65 per cent R. H. at from 72° to 89°F.). The percentage of flowers forming pods decreased when relative humidities exceeded 70 per cent or were less than 20 per cent.

Locality effects.—No effects of either climate or soil upon fruit production in the various regions of Haiti were detected which could be definitely dissociated from their effects upon overall plant growth. High rainfall and acid, leached soils combined to cause stunted growth or in some cases to prevent growth altogether. Where good growth did occur in the wetter regions (as on sandy, alluvial, river bottom soils) fruit and flower production of *C. grandiflora* approached that at Gonaives. No tests were made with either *C. madagascariensis* or the hybrid at the various subplots in the present investigation. Observations of the author in California, Arizona, and Florida lead to the generalization that *C. madagascariensis* sets most fruits in areas of high humidity (as in the coastal belt of California, the Miami region of Florida and the Port-au-Prince region of Haiti) while *C. grandiflora* is most fruitful in the drier areas (Arizona and the dry region of Haiti). This ecological differentiation of fruiting behavior in the two species is reflected by their areal distribution under natural conditions in Madagascar. *C. madagascariensis* is found on the west and northwest coasts, from Tulear to Diego Suarez, where the rainfall is between 37 cm. and 100 cm. per year, while *C. grandiflora* is confined to the southern tip of the island, between Tulear and Fort Dauphin, where the rainfall is only 30 cm. per year (Vacher, 1907).

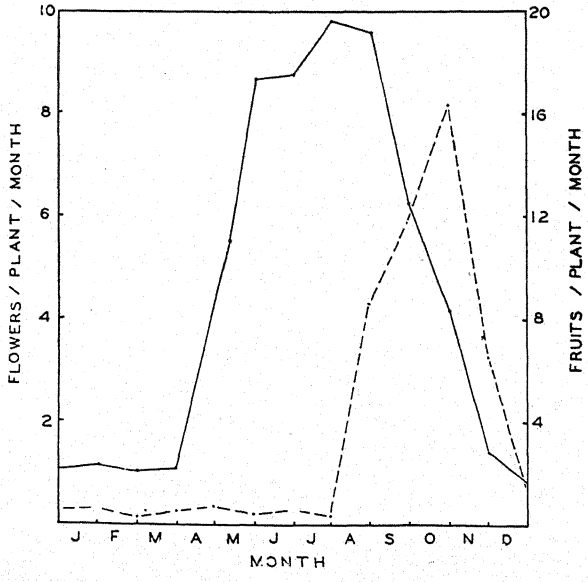


Fig. 5. Flower and fruit production by plants of a grafted clone of *C. madagascariensis* in 1944. Dotted line, fruits per plant per month; solid line, hundreds of flowers per month.

Spacing effects.—The influence of plant spacing upon fruit production in *C. grandiflora* was investigated by an experiment in which nine densities, varying from 540 to 14,300 plants per acre, were used. Randomized one-eighth acre plots were set up in triplicate for each spacing. Fruit counts were made when the plants were 19 months old. The results are shown in fig. 7. Maximum fruit production per plant occurred at the spacing of 9′ × 9′ or a density of 540 plants per acre. Highest acre yields of fruits were obtained with a spacing of 30″ × 9′ or 1,940 plants per acre. The maximum number of inflorescences and the greatest efficiency, in terms of numbers of fruits set per inflorescence, were both found at the greatest spacing distance. The crowns of the plants in these plots were 6 feet high and averaged 5 feet in diameter. Since the distance between plants was 9 feet, both in the row and between rows, there was no actual contact of the above-ground portions of the plants. Because of this it is believed that greater spacing distances would not have resulted in appreciably greater fruit production per plant. Carlson (1932) reported that the yield of alfalfa seeds per plant increased more than three-fold as the number of plants per acre decreased from 15,860 to 4,545, although the total yield per acre showed no significant difference over this range. It is to be expected that the yield per acre would decrease at spacing distances both larger and smaller than those used by Carlson.

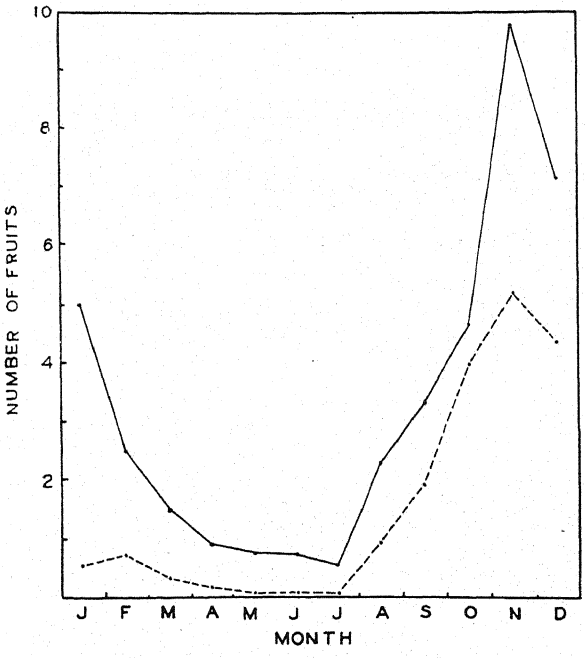


Fig. 6. Reproductive efficiency of the flowers of grafted clones of *C. grandiflora* (solid line) and *C. madagascariensis* (dotted line) in terms of the total number of fruits per plant expressed as a percentage of the total number of flowers.

Effect of grafting.—Evidence concerning the influence of root systems on flower and fruit production was obtained by means of experiments with reciprocal grafts between the two species. When *C. madagascariensis* whip scions were grafted on *C. grandiflora* stocks the resulting plants tended to flower more quickly and more abundantly than non-grafted controls. In one instance flower buds were visible 10 days after the graft was made and open flowers were present by 32 days. Fruit production was also stimulated by grafting *C. madagascariensis* on C. *grandiflora* stocks. The reverse effect was

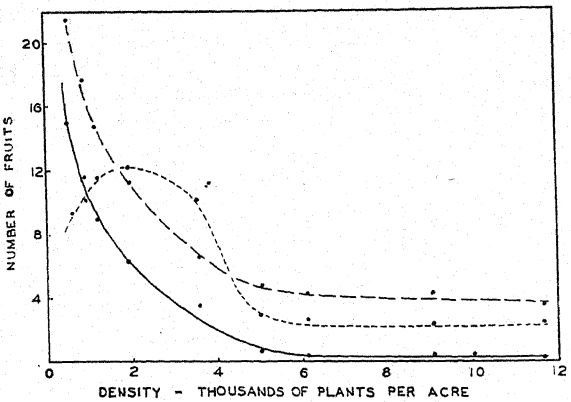


Fig. 7. Relation between number of fruits per plant (solid line), number of fruits per acre (dotted line), number of fruits per inflorescence (dashed line) and actual number of plants per acre for *C. grandiflora*. Figures on the ordinate indicate fruits per plant, thousands of fruits per acre, and fruits per hundred inflorescences.

produced by the reciprocal graft. That is, both flowers and fruits were inhibited by grafting *C. grandiflora* scions on *C. madagascariensis* stocks. In one experiment involving ten plants of each graft type (table 1), 17.1 per cent of the inflorescences of *C. grandiflora* on *C. grandiflora* stocks set fruit. All plants which had *C. grandiflora* roots, both grafted and non-grafted, had more flowers and more fruits than any of the plants with *C. madagascariensis* roots, regardless of the specific nature of the tops. The scions were from clonal material while the stocks were siblings of each species, but similar results were obtained in other experiments with plants of different origins. Both scion plants and stock plants were of the same age and the grafts were made in the same field plot under conditions of uniform environment and culture. The amount of vegetative growth of all grafted and control plants was closely comparable. Exhaustive tests of latex yield and quality showed no differences due to grafting. The observed differences in reproductive behavior appeared to be due solely to some unknown physiological interaction of stock on scion. It might be assumed that the roots of *C. grandiflora* produce some substance which is of immediate importance in the development of flowers and production of fruits, while *C. madagascariensis* roots are deficient in the substance. However, none of the reproductively active substances reported in recent studies (florigen, vernalin, ethylene) have been connected with root metabolism.

There are many instances in the horticultural literature of the effect of reciprocal grafting on fruit production. Very often one member of a pair causes a reduction in fruit yield of the other because of "incompatibility" or overall decreased vigor in the grafted plant, but this is not universally true. Stephens (1943) reported a direct effect of cotton stocks on the reproductive behavior of the scions which was independent of graft vigor and hence was very similar to the situation in *Cryptostegia*. In an experiment in which Triumph (an annual variety of *Gossypium hirsutum*) was reciprocally grafted with V-135 (a semi-perennial variety of *G. bardadense*) it was found that the total yields of bolls per acre were greatest when V-135 was the stock, regardless of the variety used for scion. On the other hand, the Triumph stocks induced early fruiting in the scions, while V-135 stocks caused a retardation of the maximum fruiting period. Thus the existence of a root effect upon the fruiting response of tops appears to be widespread in the flowering plants. Further detailed physiological investigation of the phenomenon should add materially to our knowledge of the factors involved in the initiation of the reproductive state in plants.

Pollination.—The complicated nature of the internal flower structure indicates a high degree of modification for insect pollination. There is no possibility for self-pollination in the sense of pollen transfer from anther to stigma of the same flower without the help of external agencies. The flower

TABLE 1. *Flower and fruit production by reciprocal interspecific grafts. Determinations made in October at graft age of 5½ months.*

Type of graft	Number of inflorescences per plant	Number of fruits per plant	Per cent of inflorescences setting fruit
C. madagascariensis control	27	0.6	2.2
C. madagascariensis on *C. madagascariensis*	30	0.5	1.6
C. madagascariensis on *C. grandiflora*	47	5.0	10.6
C. grandiflora control	45	7.1	15.8
C. grandiflora on *C. grandiflora*	41	7.0	17.1
C. grandiflora on *C. madagascariensis*	1	0	0

structure is such that only insects with certain definite physical characteristics can effect pollination. The body of the insect must be small enough to enter into the narrow corolla tube (11.2 mm. in diameter in *C. grandiflora*) and its mouth parts long enough to extend from the corolla gland barrier to the nectary (16.2 mm.). Insects too large to penetrate to the barrier must have mouth parts of sufficient length to compensate for the added distance. At Gonaives the only common pollinators are skipper butterflies, of which *Atalopedes mesogramma* (Latr.) and a species of *Lerodea* (*Hesperiidae*) are the most frequent visitors (Knight, 1944). Both of these species are sufficiently small to penetrate the corolla tube and both have coiled mouthparts 17 mm. long. The pupae of the *Atalopedes* species were found in numbers beneath the peeling, stringy bark of the mesquite tree (*Prosopis juliflora* P. DC.), which was common in the vicinity of all actively spreading colonies of *Cryptostegia* in Haiti. The common sulfur-colored butterfly (*Phoebis sennae* L., *Pieridae*) visits the flowers and is especially noticeable during the annual southward mass migrations in June. Its 25 mm.-long mouth parts are not long enough to entirely make up for the increased distance caused by its large body size but occasional instances of translator removal by this species have been noted.

Since the flowers (including stigma and style) abscise within 24 hours of their opening and many of them within 6 hours, it follows that pollen germination and pollen tube growth must be sufficiently rapid to allow complete penetration into the ovary within that time. There was no evidence that pollination provided any stimulus to inhibit the formation of the intra-floral abscission layers. Artificial germination of pollen can be obtained within two hours on 10 per cent sucrose agar or by planting on the stigmatic surfaces of excised flowers. Pollen is capable of germinating 36 hours prior to anthesis; the stigmatic surface is receptive 24 hours before flower opening.

Positional effect of inflorescence.—The number of flowers produced on a single inflorescence varied with season of the year and with plant vigor. Very young or stunted plants often had solitary flowers, while mature plants in open spaces with favorable moisture conditions produced up to 25 flowers per inflorescence in *C. grandiflora* and 68 in *C. madagascariensis*. All of the flowers were by no means equal with regard to potentiality for fruit set. Determination of the position of the fruits on those inflorescences on which at least one fruit was produced showed that the most efficient flowers were the ones which opened first, with efficiency falling off very rapidly for the later flower pairs at succeeding inflorescence levels (table 2). These determinations were made on fruits of mature size. Actual fruit set, in the sense of pollination and initial ovary elongation, was not so low for the later flowers, but such fruits did not continue normal development. Valleau (1918) reported a similar phenomenon in an exhaustive study of sterility in the cultivated strawberry. The inflorescence of this plant is a dichasial cyme like that in *Cryptostegia*. Reproductive efficiency dropped from an average value of 98.7 per cent in the first flowers to a low of 16.6 per cent in flowers at the fifth inflorescence level in the case of 10 open-pollinated varieties and from 88.6 per cent in the first to 31.0 per cent in the fourth flowers in 125 self-pollinated varieties. Fruit size and number of achenes per fruit also decreased at the later levels. Valleau determined that the decreased fruitfulness was not due to absence of pollination nor to pollen sterility; he attributed it to "pistil sterility." Certain observations in *Cryptostegia* lead to the conclusion that the increasing numbers of abscission layers at the joints in the branches of mature inflorescences are in part responsible for the low efficiency through interference with the conduction of necessary substances from the lower part of the plant.

TABLE 2. *Reproductive efficiency of flowers in relation to position on inflorescence.*

Inflorescence level	Percentage of flowers setting fruits		
	C. grandiflora	*C. madagascariensis*	F_1 hybrid
	%	%	%
1	38.7	31.8	11.9
2	17.3	11.3	9.7
3	8.3	2.3	5.0
4	4.7	2.4	3.7
5	0.5	1.7	1.2
6	0	0.8	0.7
7	0	0	0.3
8	0	0	0.2

Fruit development.—The rates of fruit development were quite similar in the two species, the average time between flowers and ripe, open fruits being 173 days and 180 days for *C. grandiflora* and *C. madagascariensis*, respectively. No detailed study of sterility factors was undertaken in the present investigation but it is known that several different types exist. Many ovaries abscised 10 or 12 days after pollination, the number being greatest at those seasons which had the lowest total fruit production. Similar periodicities in fruit shedding were reported by Adriance (1931) for pecans and by Murneek (1933) for apples. These have been interpreted by some investigators as due to a lack of fertilization or to early embryo degeneration with consequent failure in the maintenance of growth substance supplies adequate for abscission-layer inhibition. Often one follicle would turn black and abscise at this same 10 to 12 day period while the other follicle continued development and matured normally. The explanation for this type of sterility appeared to be the lack of a sufficient amount of pollination, since it was noted that the single follicles developed from flowers which received a pollen mass on only one stigma facet, so that pollen

tube growth was confined to a single style and hence to a single carpel.

The production of full-sized, regular-term fruits with complete or partial absence of fertile seeds was found in *C. grandiflora* only in certain races which had completely or partly albino flowers. Over 50 pods of the completely albino *C. grandiflora* clone (no trace of anthocyanin in any part of the plant) were examined without finding any good seeds. In several clones whose flowers had only a faint tinge of color at the corolla base, the number of good seeds varied from 8 per cent to 50 per cent

TABLE 3. *Weight of fruits and weight and number of seeds.*

Plant	Air dry weight of ripe fruits	Air dry weight of ripe seeds	Number of seeds per fruit
	gm.	mg.	
C. grandiflora	23.86	9.04	668
C. madagascariensis	13.84	13.10	194
F_1 hybrid	24.00	12.69	470

of the total. In normal, fully colored *C. grandiflora* plants, the number of seeds with viable embryos varied from 95 per cent to 98 per cent of the total which were visible in the ripe pod. This total number of wholly or partly developed ovules, however, represented only 89 per cent of the total number of ovules originally present, as determined by ovule counts in the ovaries of open flowers.

The absolute number of seeds per fruit varied considerably from season to season, with the greatest number occurring in the autumn fruiting peak. Average numbers and weights of seeds for the two species and the F_1 hybrid are shown in table 3, along with dry fruit weights.

The season of maximum seed dispersal in the Gonaives area is that of the dry winter months, although some seeds are ripe at all times of the year. When mature, the follicles split longitudinally along their upper face, thus allowing the seed plumes to be opened by the wind and carried off.

Reproductive energy.—Some concept of the amount of energy devoted to reproduction in this plant can be gained by a study of the total amount of flowers and fruits produced per acre. Flower and fruit counts were made in 1944 on grafted plants of both of the species and the hybrid at a spacing of 1.5′ × 8′ or 3,000 per acre. The plants were nine months old at the beginning of the year and averaged 30″ in height and 44″ in greatest width. At the end of the year they were 42″ × 56″. Flower and fruit numbers per acre and total dry weight of flowers and fruits are shown in table 4. Over two tons of flowers and nearly ten tons of fruits were produced by one acre of the grafted hybrid plants in the year. This was 101 per cent of the total dry weight of the entire vegetative tops (12.1 tons) as determined by sampling at the end of the year. Since the top weight at the beginning of the year was 3.6 tons per acre the annual increment of vegetative growth was 8.5 tons. Thus the annual weight of reproductive material was 145 per cent of the annual weight of vegetative material produced.

Comparable studies on other plants, mostly temperate zone crops with short reproductive seasons, have given values much lower than those for *Cryptostegia*. According to Grainger (1943), flowers ordinarily constitute about 5 per cent of the total dry weight of a plant. Anderson (1944) reported that the flower heads of timothy were 5.7 per cent of the total dry weight. This ratio is far less than that of the *Cryptostegia* hybrid, where flowers amounted to 29.8 per cent of the total dry weight of the annual vegetative material. The difference is in part attributable to the everblooming characteristics of *Cryptostegia*.

SUMMARY

Investigations of fruit production in two species of *Cryptostegia* and their F_1 hybrid were carried out in Haiti in 1943, 1944 and 1945. Both flowers and fruits were produced throughout the year but maximum flowering occurred in the summer. The greatest number of fruits in *C. madagascariensis* was set in the autumn months, while optimum fruit-set in *C. grandiflora* and the hybrid occurred 2 months after the main peaks in the bimodal rainy season.

No direct effect upon reproduction in *C. grandiflora* could be found for a wide range of soil and moisture conditions in various parts of Haiti. The maximum number of fruits per plant and the greatest reproductive efficiency of the flowers both occurred at field spacings of 540 plants per acre. Highest acre yields of fruits were provided by plant densities of 1,940 per acre. Reciprocal grafting

TABLE 4. *Flower and fruit production per acre per year. Grafted clones—3000 plants per acre.*

Character	*C. grandiflora*	*C. madagascariensis*	F_1 hybrid
Number of flowers per acre per year.....	8,061,600	17,365,500	9,265,500
Number of fruits per acre per year.....	182,790	146,500	369,600
Dry weight of flowers, in pounds.....	4,256	5,728	5,096
Dry weight of fruits, in pounds.....	9,192	4,462	19,514
Total dry weight of reproductive material, in short tons	6.7	5.1	12.3
Ratio—dry weight fruits to dry weight flowers	2.16	0.78	3.84

experiments indicated a partial control over reproduction by the root systems. All plants, both grafted and non-grafted, that had *C. glandiflora* stocks exceeded plants with *C. madagascariensis* stocks in flower and fruit production, irrespective of the specific nature of the scions.

Pollination was brought about largely by butterflies. The first flowers to open on a given inflorescence showed the greatest reproductive efficiency in terms of numbers of flowers per mature fruit. Efficiency of later flowers fell off very rapidly. Average annual figures for fruit set showed that one fruit was set for every 44 flowers in *C. grandiflora,* one for every 119 flowers in *C. madagascariensis,* and one for every 25 flowers in the F_1 hybrid.

Fruits of both species reached maturity in 170 to 180 days. The only cases of natural parthenocarpy found were in certain clones characterized by the absence of anthocyanin from all plant parts. In most instances, sterility factors were expressed by the early termination of fruit development; such failures were most numerous within the first 10 or 12 days following pollination.

Dry weight production of flowers and fruits amounted to 12.3 tons per acre per year for hybrid plants grafted on *C. grandiflora* stocks. This was 145 per cent of the total vegetative material produced in the year.

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THE ABSORPTION AND REFLECTION SPECTRA OF LEAVES, CHLOROPLAST SUSPENSIONS, AND CHLOROPLAST FRAGMENTS AS MEASURED IN AN ULBRICHT SPHERE [1]

G. S. Rabideau, C. S. French, and A. S. Holt

The green supernatant extract obtained by centrifuging a chloroplast suspension which has been disintegrated in a supersonic field was found by French, Holt, Powell, and Anson (1946) to evolve oxygen when irradiated in the presence of suitable reagents. In order to obtain further information on the nature of the photochemical reaction involved, it was desirable to determine the absorption spectrum of the extracts. The extracts of chloroplast constituents are colloidal dispersions and may scatter visible and near visible radiant energy to varying degrees as is also the case with intact leaves to a far greater extent. Consequently it is impossible to obtain the absorption spectra of the pigments by direct transmission methods with a spectrophotometer. The following report concerns the absorption spectra of supersonic treated chloroplast suspensions, untreated chloroplast suspensions and intact leaves obtained by means of an Ulbricht sphere grating spectrophotometer.

Seybold and Weissweiler (1942a, 1942b) made an extensive series of absorption and reflection measurements of leaves, algae, chloroplasts, and leaf pigments in vitro using the Hardy recording spectrophotometer, with which our present apparatus is essentially equivalent in regard to the measurement of diffuse light. Mestre (1935) and Seybold and Weissweiler (1942a) have discussed the optical principles involved in the measurement of diffuse reflection and transmission of light. Katz and Wassink (1939) have presented the absorption

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TABLE I. *Glass filter combinations, photocell response, and per cent of stray light for different wavelength settings.*

mμ	Photo-tube	Order	Slit mm.[i]	Lamp current amps.	Filters[e]	Test filter	Effective stray light	Photocell response volts
1000	917	1	..	..	87, 255	....	...	0.03
900	917	1	..	..	87, 255	1 cm. $FeSO_4$[a]	...	0.59
850	917	1	..	..	87, 255, 512	1 cm. $FeSO_4$	...	0.34
820	917	1	..	..	88A, 255	....	...	....
800[b]	917	1	..	..	88A, 255	....	...	0.04
					1 cm. $FeSO_4$			
780[g]	917	1	..	..	88A, 255	....	...	....
					1 cm. $FeSO_4$			
770	917	1	..	..	88A, 255, 512	....	...	....
760	917	1	..	..	88A, 255, 512	87	...	....
750	917	1	1	68	RG8, VG3, 5031	87	...	0.50
740	917	1	1	68	RG8, VG3, 5031	87	1.9	1.00
730	917	1	1	68	88, VG3, 5031	87	1.4	0.60
720	917	1	1	68	88, VG3	5030	1.2	0.47
710	917	1	1	68	RG8, VG3, 512	5030	0.8	1.08
710	929	1	1	72	RG8, VG3	....	...	0.08
700	917	1	1	68	RG5, VG3, 512	88A	0.1	1.00
700	929	1	1	68	RG5, VG3	88	0.1	0.13
691Hg	929	1	3	..	RG5	....	...	....
690	929	1	1	68	RG5	....	...	0.22
680	929	1	1	68	RG5	9780	0.5	0.21
670	929	1	1	68	241[c]	....	...	0.41
660	929	1	1	68	241	....	...	0.38
650	929	1	1	70	241	RG5	< 1.7	0.36
640	929	2	1	70	242	....	...	0.11
640	929	1	1	68	242	....	...	0.50
630	929	2	1	70	243[f]	....	...	0.19
630	929	1	1	70	243[f]	RG5	0.3	1.00
620	929	2	1	70	2412	241	1.0	0.25
610	929	2	1	70	RG1	243[h]	1.0	0.19
600	929	2	1	70	2424, 9780½T	5013	0.8	0.25
590	929	2	1	70	2424, 9780½T	512	0.9	0.22
580	929	2	1	70	3480, 9780½T	512	0.6	0.67
578Hg	929	2	1	..	3480, 9780	512, 2418	0.1	0.64
570	929	2	1	70	3482, 9780, VG3	5030	0.2	0.10
560	929	2	1	70	3482, 9780, 512	5030	0.5	0.91
550	929	2	1	70	3484, 4305, 512	5543	0.0	0.38
546Hg	929	2	1	..	430, 512, 3484	3480	0.8	0.34
540	929	2	1	70	3486, 4305, 401½T	5543	0.0	0.13
530	929	2	1	70	3486, 430, 401½T	5543	0.3	0.17
520	929	2	1	70	VG3, 430, 3384	5543	< 1.0	0.33
510	929	2	1	70	GG11, 5031, 401½T	511	0.0	0.48
500	929	2	1	70	512, 45, 3385	5113½T	0.0	0.57
491Hg	929	2	2	..	3385, 512, 5030	3384	...	....
490	929	2	1	70	3385, 512, 5030	3384	1.8	0.17
480	929	2	1	70	3387, 5030	5850	5.1	0.77
470	929	2	1	70	3387, 5030	3385	6.0	0.16
460	929	2	1	70	3389, 5030, 5543	3385	0.3	0.93
450	929	2	1	70	3389, 5543	3385	0.3	0.90
440	929	2	1	70	3389, 511, 5543	3387	0.0	0.14
436Hg	929	2	1	..	3389, 511, 5543	3387	0.5	0.56
430	929	2	1	70	3389, 5850, 5543	3387	0.5	0.17
420	929	2	1	74	3060, 511, 5850, 512	3387	0.0	0.06

[a] Saturated $FeSO_4$ in 25 per cent H_2SO_4.
[b] A 11.5 cm. water filter was used at all shorter wavelengths.
[c] Filters # 87, 88, and 88A are Wratten filters, RG1, RG5, RG8, VG3, GG11, and UG1 are Jena. All others are Corning.
[d] Plus a cuprammonium solution whose concentration was adjusted visually to give a suitable transmission.
[e] 241 H.R. Pyrometer red ("36 per cent") (4.95 mm.).
[f] 243 H.R. Signal red ("157 per cent") (3.4 mm.).
[g] A 2.6 cm. filter of saturated $FeSO_4$ in 25 per cent H_2SO_4 was used for all shorter wavelengths.
[h] 243 H.R. Signal red ("145 per cent") (5.15 mm.).
[i] dispersion: 8.1 mμ/mm. in first order and 4.05 mμ/mm. in second order.

TABLE 1. *Concluded.*

mμ	Photo-tube	Order	Slit mm.[i]	Lamp current amps.	Filters[e]	Test filter	Effective stray light	Photocell response volts
410	929	2	1	74	385, 511, 5070, 512	5850	4.0	0.07
405Hg	929	2	2	..	3060, 511, 597	3387	1.3	0.04
400	929	2	1	70	385, 5070[d]	3387	3.9	0.08
390	929	2	2	74	385, 5970[d]	3387	<3.2	0.12
380	929	2	2	74	5970[d]	3387	1.6	0.09
370	929	2	2	76	5840, UG1	3389	5.0	0.11
365Hg	929	2	4	..	5840, 9780½T	3060	0.2	0.21
360	929	2	4	76	5840, UG1	385	<10.0	0.06

curves of Chlorella cells disintegrated by grinding with carborundum. Smith (1938) measured the absorption spectrum of a cell-free pigment suspension and found that positions of the absorption maxima coincided with those obtained with an intact leaf. Katz and Wassink showed that in an aqueous mixture of fresh egg albumin and colloidal chlorophyll the absorption maxima were shifted to coincide with those of intact leaves.

In general, our measurements of leaf spectra confirm the data of Seybold (1932a, 1932b, 1933a, 1933b) and Seybold and Weissweiler (1942a, 1942b, 1943) and extend the curves over a broader spectral range. A band width of about 10 mμ was used over most of the visible range. The authors are not aware of any study using an integrating sphere with a band width narrow enough to give information as to the precise position of the chlorophyll absorption maximum in the red region. The dangers of attaching too much significance to small differences in band position in various leaves have been discussed by Mestre.

MATERIALS AND METHODS.—Light from a tungsten lamp was passed through a large grating monochromator described by French, Rabideau, and Holt (1946). The light coming from the exit slit was reflected off a mirror into the Ulbricht sphere. A lens in the bottom of the sphere focused the light on the absorption vessels which were suspended in the center of the sphere as shown in fig. 1. The construction and use of the Ulbricht sphere has been described by French and Rabideau (1945). The measurement of the light intensity in the sphere was accomplished by means of a photoelectric cell, a Leeds and Northrup thermionic D.C. amplifier, a galvanometer, and a potentiometer. To cover adequately the wavelength region investigated, two phototubes were used: (1) an R. C. A. type 929 with a maximum sensitivity in the blue region, used for wavelengths below 690 mμ, and (2) an R. C. A. type 917 with a maximum sensitivity in the near infrared region used for wavelengths above 690 mμ. These phototubes were mounted in a moisture-proof lucite housing on the lower half of the sphere and were shielded from direct illumination by the light beam and from light reflected from the vessels. An external switching system allowed either phototube to be connected.

Two metal rings held the leaf sections or absorption vessels and were attached to an arm actuated by a lever outside the sphere. The incident intensity reading, I_o, was taken with the samples in the sphere but not in the beam. The transmitted intensity, I_x, was measured when the uncovered vessel or leaf was brought into the light beam as shown in fig. 2. The reflected light was measured by covering the second absorption vessel or leaf section with a piece of black felt cloth coated with lampblack. The top of this disk was covered with Al_2O_3 in collodion similar to the coating on the inside of the

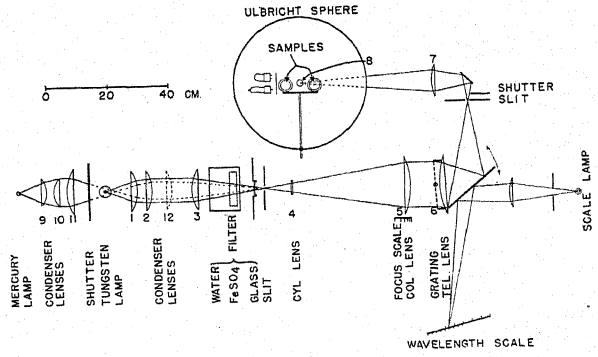


Fig. 1. A diagram of the Ulbricht sphere and the monochromator.

sphere. The black disk was found to have a constant reflection of 4–5 per cent between 450 and 850 mμ. The reading obtained with the uncovered sample in the beam represents the sum of the transmitted and reflected light (R + T) and is expressed as a fraction of the I_o reading. In this way, the absorption was given by Seybold (1932b) as:

$$A = 1 - (R + T),$$

where A is the fraction absorbed, R the fraction reflected, and T the fraction transmitted.

In order to avoid reflection of the light out of the sphere along the path of its entrance, the angle of incidence was adjusted to about 10°. The chloroplast suspensions were held in the sphere in Carrel culture flasks of 3.5 cm. diameter and 1.03 cm. thickness. These flasks had upturned necks, so that measurements could be taken in a horizontal position. The intact leaves were measured as circular

sections 36 mm. in diameter held in place by thin metal rings. The sections were cut with a tool similar to a corkborer. A small amount of water was placed around the ring to keep the leaf section from drying out during the measurement.

To reduce stray light to a minimum, baffles were placed around the base of the Ulbricht sphere and around the monochromator slits. As a stray light test in the sphere, the vessel was replaced by a mirror held at an angle which would reflect the beam out of the sphere when the top hemisphere was lifted slightly. Suitable black cloth baffles were so located as to prevent any stray light from entering the parted hemispheres. When the shutter was opened, no measurable deflection was noted, showing the absence of stray light from any part of the beam which was not incident upon the sample.

The amount of stray light coming through the monochromator was determined according to the method of Cary and Beckman (1941) by choosing glass filters to absorb completely the wavelength region isolated by the monochromator and its filters. The reading with the filters, expressed as a fraction of the reading without the cut-off filters, was taken as the per cent of stray light of wavelength other than that of the isolated region. These measurements and the filters used to remove light of other wavelengths are found in columns 7 and 8 of table 1. In over 70 per cent of the wavelength settings, the effective stray light as measured by the photocell is 1 per cent or less, and in only four cases did the value reach 5 per cent.

In order to check the over-all accuracy of the apparatus, a comparison was made between the previously described instrument and the Beckman photoelectric spectrophotometer model DU with: (1) a solution of xanthophyll (American Chlorophyll Co.) of 2.2×10^{-6} M. in acetone, and (2) a 1.35×10^{-6} M. aqueous solution of methyl violet (Grübler) buffered with 1×10^{-3} M. potassium biphthalate. In order to compare the Ulbricht sphere results with those from the Beckman apparatus, the apparent transmission measurements obtained with the integrating sphere had to be corrected for the reflection of light from the front surface of the vessel.[2] These data are shown in table 2.

[2] The calculation of the true absorption of solutions or suspensions from measurements in glass vessels in the sphere: In the measurement of the absorption of light by a colored solution, the usual spectrometric practice is to compare the light intensity after the beam has passed through an absorption vessel containing the colored solution with the same beam after it has passed through an identical absorption vessel containing the colorless solvent. In this way, losses due to reflection and absorption of the vessel walls are compensated. However, when the vessel is placed in the center of the Ulbricht sphere and inclined slightly from the normal, the above procedure is no longer applicable since reflected and scattered light are included in the measurements of the light intensity after absorption. Corrections for the reflection at the surfaces of the pyrex vessel and for the absorption by the vessel itself have therefore been introduced. The symbols are:

I_o = The intensity incident on the first surface of the vessel.
I = The intensity incident on the second surface of the vessel.
R_1 = The fraction of I_o reflected from the first surface.
R_2 = The fraction of I_o reflected from the second surface.
R_2' = The fraction of R_2 that emerges from the vessel.
t_o = The transmission of each of the vessel windows determined with water in the vessel, where transmission $= \frac{y}{y_o}$, y_o being the intensity incident on the first vessel surface and y the intensity of the beam emerging from the other vessel surface.
t = The transmittance of the solution, where transmittance $= \frac{x}{x_o}$, x_o being the intensity of the beam entering the solution and x the intensity of the beam reaching the opposite interface.
r_1 = The fractional reflection at the air-pyrex interface.
r_2 = The fractional reflection at the pyrex-water interface.
t_1 = The transmittance of 1 mm. of pyrex.
T = The light emergent from the second vessel surface, as a fraction of I_o.
M = The total light with the vessel in the beam expressed as a fraction of I_o.

The derivation of the reflection correction is as follows:

(1) $R_1 = I_o (r_1 + r_2 t_1 - r_1 r_2 t_1)$
(2) $R_2 = I_o t_o t (r_2 + r_1 t_1 - r_1 r_2 t_1)$
(3) $R_2' = I_o t_o^2 t^2 (r_2 + r_1 t_1 - r_1 r_2 t_1)$
(4) $t_o = (1 - r_1)(1 - r_2) t_1$
(5) $I = I_o t_o t$
(6) $T = I_o t_o^2 t$
(7) $M = T + R = T + R_1 + R_2'$
(8) $M = I_o t_o^2 t^2 (r_2 + r_1 t_1) + I_o t_o^2 t + I_o (r_1 + r_2 t_1)$.

By substituting in equation (7) and simplifying, the quadratic equation (8) was obtained in terms of t. At 589 mμ the index of refraction of pyrex glass is given as 1.474. The values for r_1 and r_2 calculated for normal incidence at this wavelength were 0.0367 and 0.0025 respectively. The transmission values for 2 and 5 mm. thicknesses of pyrex are given as 92 per cent and 90.5 per cent respectively, from which the transmittance of a 1 mm. thickness of pyrex was calculated to be 99.5 per cent. Evaluating the numerical terms in equation (8) we obtain:

(9) $M = 0.0365\, t^2 + 0.9141\, t + 0.0391$.

A curve was plotted from equation (9) and used to convert the measured values of M in the Ulbricht sphere into values of true transmittance, t.

A comparison was made between some absorption measurements made with tungsten and with mercury light sources, to ascertain whether or not the wider wavelength band isolated from the tungsten lamp gave different results from those obtained with monochromatic light. A chloroplast suspension containing about 0.01 mg. chlorophyll per ml. was used for these measurements. The results are given in table 3.

RESULTS AND DISCUSSION.—Absorption and reflection spectra were measured at 10 mμ intervals with the following species of leaves: *Ficus* sp., *Tilia Americana, Dolichos* sp., *Lactuca sativa, Brassica oleracea var. capitata, Ananas* sp., *Impatiens biflora,* and *Ipomea batatus.* The circular sections were cut from the leaves just prior to measurement

and were placed with the upper surface toward the incident light. Only pairs of leaf sections having absorption values which agreed within 1 per cent were used. The results of these measurements are shown in curves A-H in fig. 3 and 4. These curves show a reflection maximum and an absorption minimum at 550 mμ and an absorption maximum at about 670–80 mμ. A second absorption maximum is found in the blue region but is not distinct. In general, there is high reflection and low absorption in the red and infrared region. This is also shown by the data in fig. 7. The thick leaves showed a greater absorption than did the thin leaves. Leaves such as cabbage and lettuce, which were less green than the others, were found to reflect more of the incident light.

The absorption and reflection spectra were determined for chloroplast suspensions prepared in the manner described by Kumm and French (1945). The amount of chloroplast-chlorophyll in the suspensions was determined photometrically and equal dilutions were used for the two absorption vessels. Curves I, J and K in fig. 4 show the results of the absorption and reflection measurements for such suspensions. The curves show the same location for the maxima and minima as do the intact leaf sections, but the peaks are more pronounced. This is more evident when a comparison is made between curve H, the intact *Dolichos* leaf, and curve I, the *Dolichos* chloroplast suspension. This comparison is not of quantitative significance, however, since the amount of chlorophyll per unit area was not equal in the two cases.

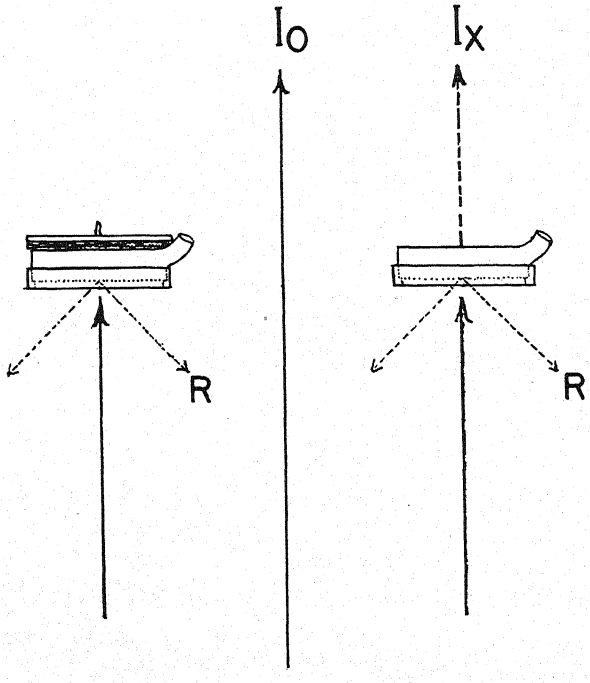


Fig. 2. A diagram of the position of the absorption vessels in the Ulbricht sphere in relation to the incident light beam.

TABLE 2. *A comparison of transmission measurements of clear solutions as made with the Ulbricht sphere and with the Beckman spectrophotometer.*

Wavelength mμ	Per cent transmission of solution		
	Beckman	Sphere	Difference
Xanthophyll			
420	59.6	58.8	—0.8
430	55.9	55.6	—0.3
440	48.0	46.8	—1.2
450	44.3	41.9	—2.4
460	52.7	49.8	—2.9
470	50.3	50.5	+0.2
480	50.4	51.0	+0.6
Methyl Violet			
540	47.4	45.8	—1.6
550	44.7	43.4	—1.3
560	42.9	43.0	+0.1
570	39.1	38.0	—1.1
580	34.2	34.8	+0.6
590	31.3	30.8	—0.5
600	35.6	34.3	—1.3
610	48.6	47.7	—0.9

Measurements were made of the absorption and reflection spectra of the supernatant fraction after centrifuging chloroplasts which had been ruptured by supersonic vibration. This was done by exposing the chloroplasts to vibrations of about 200,000 cycles/sec. from a quartz crystal immersed in an oil bath. Such preparations were made by macerating leaves in water in a Waring blendor for 1 minute, straining through a cloth and dipping a test tube containing 10–20 ml. of the strained leaf juice into the oil bath directly above the crystal for periods of 10 seconds, each exposure being followed by cooling in an ice bath. After an aggregate of 2 minutes of such exposure, the fragmented chloroplast material was centrifuged for 30 minutes in a high speed centrifuge (*ca.* 12,000 g.) after which the green supernatant was decanted and used for the spectral measurements. The results are shown in curves J and L in fig. 4. They show the same location of the maxima and minima as do intact leaves and suspensions of intact chloroplasts, but the maxima and minima are more pronounced than those of the leaves or the chloroplast suspensions. In curve J, the dotted line represents the absorption curve of the intact chloroplasts before the supersonic treatment, and the solid line the absorption curve for the supersonic supernatant fraction. An equal concentration of chlorophyll per unit area was used in each case.

The leaf curves vary considerably from each other partly because of mere differences in thickness or total pigment content. To provide a more rational basis for a comparison of these curves, we have replotted the data as log density ($\log \log \frac{I_o}{I_x}$) against wavelength. This should give a curve of a

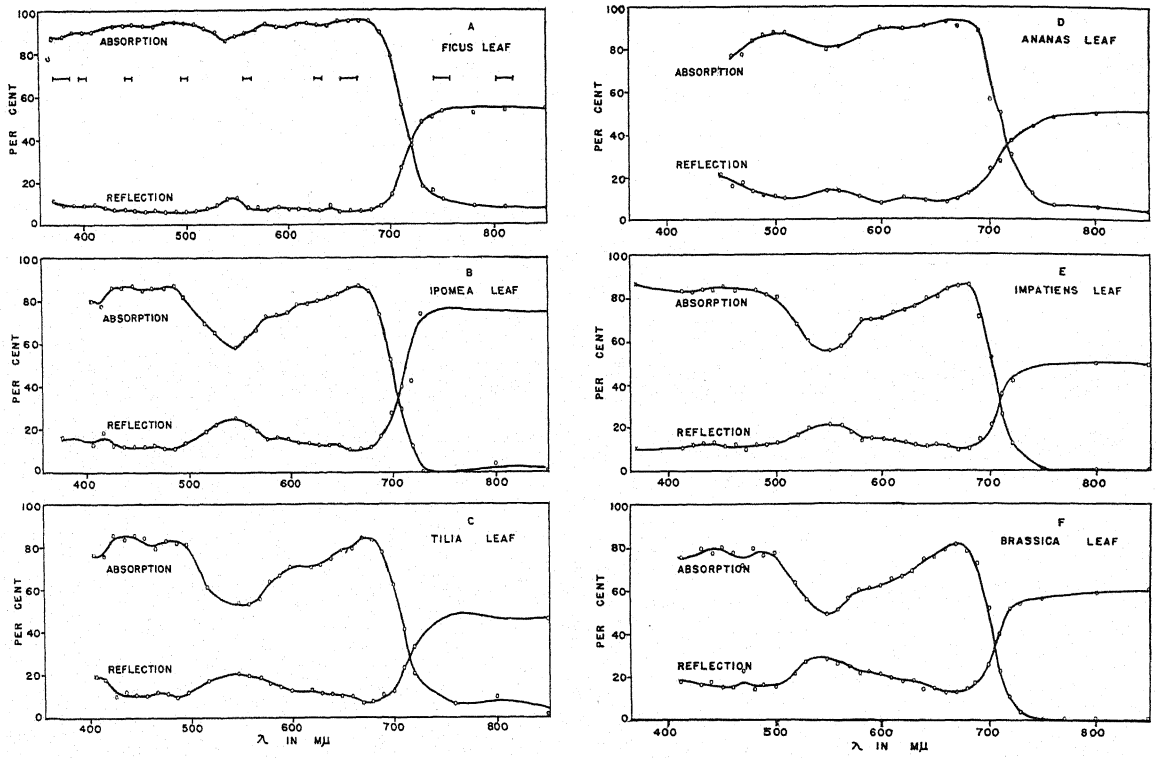


Fig. 3. The absorption and reflection curves of leaves as measured by the Ulbricht sphere.

characteristic shape which is independent of the concentration of the substance under investigation. Thus the shape of such curves is determined by the nature of the absorbing substances in the leaves and should be independent of their concentrations, since the concentration determines only the position of such a curve in the vertical direction. The plots of some of these data are shown in fig. 5. To facilitate comparisons, we have brought the curves together by shifting them in the vertical direction so that

TABLE 3. *A comparison of absorption measurements made by monochromatic light from a mercury source with the narrow spectral bands isolated from a tungsten light source with the grating monochromator. The absorption vessel was 1.029 cm. in thickness and contained about 0.01 mg. chloroplast-chlorophyll per ml. All absorption measurements were made in the Ulbricht sphere in the usual manner.*

Wavelength setting	Tungsten band width	Mercury line	Order	Absorption	Error in tungsten band measurement
mμ	mμ	mμ		per cent	per cent of absorption
691	16.2		1	87.7	+ 0.5
691		690.7	1	87.3	
578	8.1		2	40.7	— 0.5
578		577.0, 579.1	2	40.9	
546	8.1		2	46.7	+ 2.9
546		546.1	2	47.5	
436	16.2		2	84.8	+ 2.0
436		435.8	2	83.1	
366	24.3		2	91.6	— 2.1
366		365.0, 366.3	2	93.4	

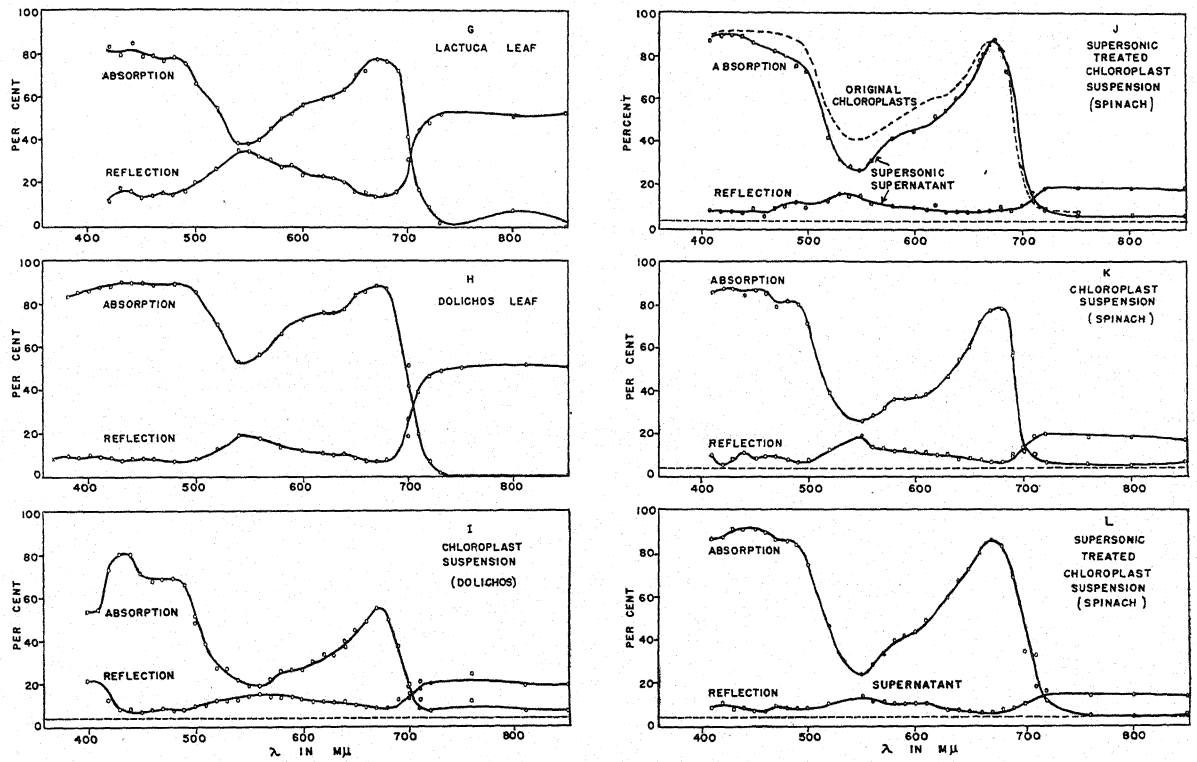


Fig. 4. The absorption and reflection curves of leaves, chloroplast suspensions, and the supernatant fraction after centrifugation of supersonic disintegrated chloroplasts, as measured with the Ulbricht sphere. The chlorophyll concentrations of the suspensions were: curve I 0.009 mg./ml., curve J, 0.0084 mg./ml. (both absorption curves); curve K 0.0084 mg./ml., curve L, 0.010 mg./ml.

they all intersect at 670 mμ. It is apparent that this procedure is not entirely adequate to characterize the absorption of the pigments present. In the chloroplast suspensions, where there is less scattering than in the leaves, the low absorption at 550 mμ is less than it is in leaves. The length of the light path in the leaf appears to be greater in the green than for the more strongly absorbed red and blue. This effect is less pronounced in chloroplast suspensions than in leaves and is still less evident in the supersonic extracts. While this method of plotting provides an improved means of comparing leaves or chloroplast suspensions having approximately the same internal light path but different pigment densities, it still does not give curves, the shape of which can be attributed solely to the nature of the pigments present. The two thick leaves used, *Ficus* and *Ananas*, were plotted separately from the family of curves of thinner leaves. They show a less striking absorption minimum. Apparently no great amount of information can be obtained from the relative heights of the various bands when we are faced with an increased length of the light path in the regions of lower absorption. The position and relative heights of the bands may be more easily compared on such graphs, however. Much interest has centered on the determination of the position of the red chlorophyll maximum in leaves. Our curves, with but one exception, throw little light on this question since the band width used was about 10 mμ, and since measurements were made only at 10 mμ intervals. However, it can be said that all curves showed the red maximum absorption to be between 670–680 mμ[3]. The supersonic supernatant material of curve J, fig. 4, was measured with the Beckman spectrophotometer at 2 mμ intervals. The results in fig. 6 show a maximum located at 678 mμ. Similar measurements for curve L gave maxima at 680, and 681 mμ. The positions of all discernible bands in the absorption curves were tabulated and the following conclusions were drawn: All but one curve gave a red maximum absorption presumably due to chlorophyll *a* at 670–680 mμ. On the short wave side of the red absorption band, a few irregularities, presumably due to chlorophyll *b* bands, were found; they were not consistently evident at any one wavelength, but were spread from 640–660 mμ. Possibly some of these could be due to the chlorophyll *a* band which has its maximum at 613 mμ in ether. Several poorly defined bands were evident in the region 600–620 mμ. *Dolichos* and *Tilia* showed these bands most distinctly. They could be due to the small maximum of chlorophyll *b* which is found at 593 mμ in ether. A very distinct band at 580–588 mμ is seen particularly in *Lactuca, Impatiens, Brassica, Ipomea,*

[3] With the possible exception of *Ananas* which gave a broad band with an apparent maximum at 660 mμ.

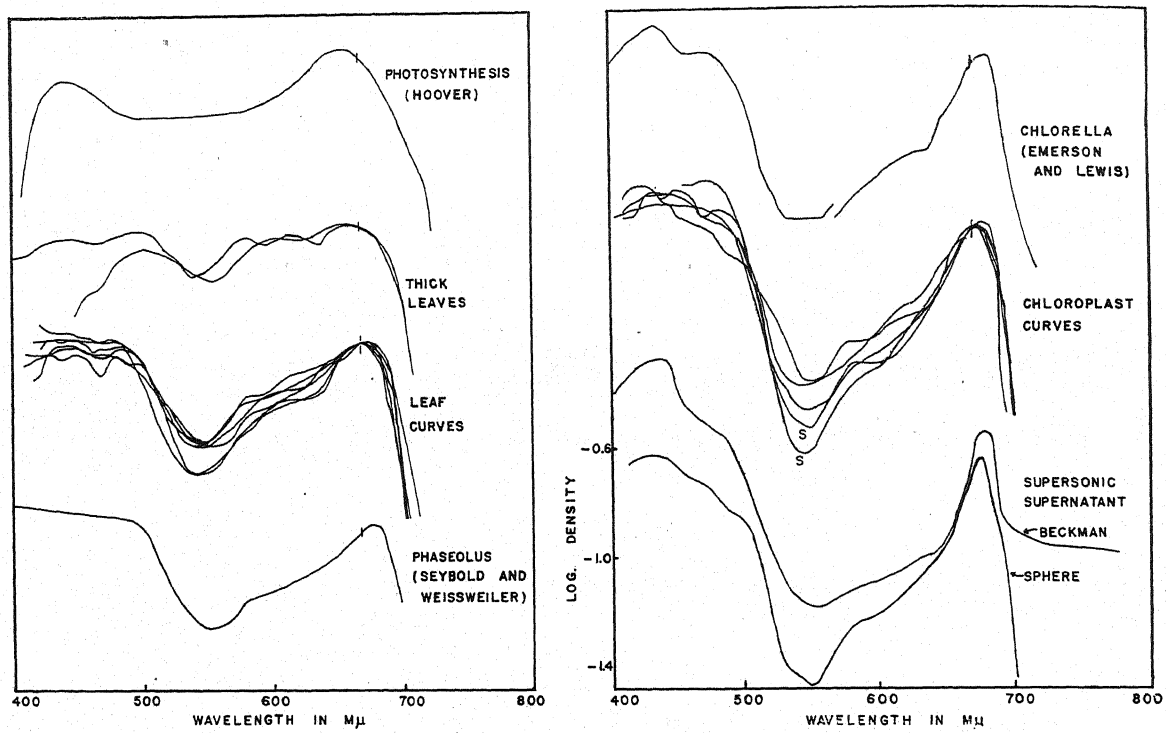


Fig. 5. The leaf and chloroplast absorption curves of fig. 3 and 4 expressed on a log density basis and adjusted to the same height at 670 mμ. Selected curves of other investigators are plotted on the same basis and are included for comparison. The Beckman and sphere curves for the supersonic supernatant are based on the data in fig. 6.

Tilia, and in all but one of the chloroplast suspensions. This could very well be the chlorophyll *a* band which is found at 576 mμ in ether. The possibility that some of these bands may also be the result of chlorophyll precursors (protochlorophyll) or breakdown products is a problem that remains to be investigated. The position of minimum absorption lies between 540–556 mμ and is probably shifted one way or another depending on the concentration of chlorophyll and of carotenoids present in the material. The bands from 480–500 mμ are presumed to be due to the carotenoid pigments and the peaks from 430–460 to the blue maxima of chlorophyll.

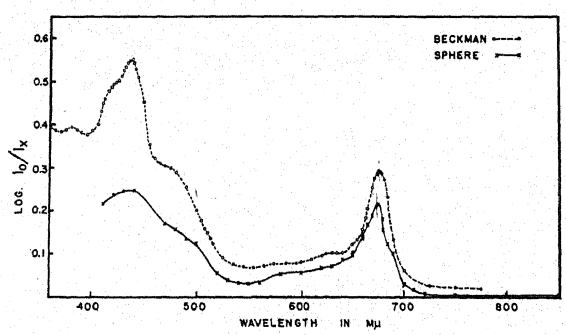


Fig. 6. A comparison of the absorption spectrum of the supersonic supernatant fraction as determined with the Ulbricht sphere and with the Beckman spectrophotometer. Reasons for the discrepancies in these curves are given in the text.

A typical curve from Seybold and Weissweiler (1942a), Hoover's photosynthesis curve (1937), and Emerson and Lewis' (1943) *Chlorella* absorption curve have been reduced to log density plots and are included in fig. 5 for purposes of comparison. In the lower right of fig. 5 is the log density plot of the data shown in fig. 6. The discrepancy between these two curves is presumed to be due to the fact that the Beckman instrument measures only the directly transmitted light while the sphere measured transmitted and scattered light. Since the preparations which were used scattered some light, close agreement could not be expected with these two methods. The Beckman curve is of value, however, in that it shows the position of the bands better than does the sphere curve, which is corrected for scattering but which was made with greater slit widths. From these data we may conclude that the treat-

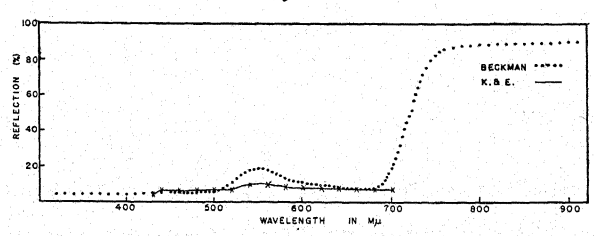


Fig. 7. The reflection of the upper surface of a lilac leaf. The data of Shull (1929) obtained with a Keuffel and Esser color analyzer are compared with measurements made with the reflection attachment of a Beckman spectrophotometer.

ment of chloroplasts with supersonic vibration does not change the positions of the absorption bands of the leaf pigments. These extracts therefore appear to contain chlorophyll in the same state but in much more finely divided particles than in intact chloroplasts.

SUMMARY

An apparatus is described in which an Ulbricht sphere was used in conjunction with a grating monochromator to measure absorption and reflection spectra.

Absorption and reflection curves are shown for eight species of leaves, several chloroplast suspensions, and the supernatant fraction after the centrifugation of chloroplasts disintegrated by supersonic vibration. The absorption curves were plotted both as per cent absorption and on the log density basis and some attempt was made to allocate the absorption maxima to certain pigments. It appears that supersonic treatment of chloroplasts does not shift the position of the pigment absorption bands.

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EFFECTS OF ZINC DEFICIENCY ON PHOSPHATE METABOLISM OF THE TOMATO PLANT [1]

Howard S. Reed

A GROWING recognition of the importance of micronutrients has accelerated investigations in many fields and has materially extended our knowledge of the physiology, biochemistry, and cytology of plants.

This paper attempts to elucidate certain phenomena exhibited by tomato plants grown in nutrient solutions of purified salts differing *inter se* in the amounts of zinc they received. The general technique of the preparation and care of the cultures has been described by Stout and Arnon (1939).

Four cultures, each containing three plants in covered pyrex glass beakers, were employed for four concentrations of zinc, namely, 0.0, 0.02, 0.1, and 0.5 parts per million. Where no zinc was added the plants were dwarfed from an early stage and eventually ceased growth; however, in the two higher concentrations they grew well and produced fruit. In the zinc deficient cultures the leaves were curved downwards, the leaflets were chlorotic, involuted, and often contained dead spots. The disrupted metabolism in the leaflet was correlated with the scarcity of plastids, production of melanotic material, and presence of calcium oxalate crystals (Reed, 1939).

STEMS.—*Distribution of phosphates.*—Fresh sections of the penultimate internodes of zinc deficient stems when tested with molybdenic reagent in watch glasses produced more blue color in an hour than those of healthy stems; moreover, the color produced by the former was diffusible. When sections were rinsed, transferred to slides, and examined under high magnification there was no significant difference in the depth of color which they retained, indicating that the former were richer in diffusible phosphate compounds or that the protoplasm was more permeable. The walls of collenchyma and central pith remained deep blue in both lots of material. Other sections of similar stems from plants of the same age were tested for inorganic phosphates with the Gomori technique which precludes their diffusion. Sections of healthy stems (fig. 1) showed an over-all brown black color. Black material indicating phosphate was observed chiefly in collenchyma walls and inner and outer phloem, but it was scarce in cambium and primary xylem. The endodermal and collenchyma cells were optically empty, though walls of the latter were dark brown. Sections of affected stems (fig. 2) contained more black material in the extra-stelar region in comparison with the healthy, though there was none in the endodermal layer. Black material was abundant in the inner and outer phloem. The pictures therefore supplement the results with molybdenic reagent and agree with the results of unpublished work on other plants showing that phosphate may be and generally is more abundant in the phloem of deranged plants than in xylem or extra-stelar tissues. The retarded development of the phloem-xylem layers in the zinc deficient stem affords another contrast which should be recognized as a clinical symptom. The parenchyma cells of the pith of the affected stem were generally larger than those of the healthy. (On the ratio of the sizes of affected cells of vegetative buds of the peach, see Reed, 1941.) Parenchyma cells contained more starch than similar cells in healthy stems, indicating an impairment of phosphorylase, hexokinase, or other enzymes in zinc deficient stems. They also contained more calcium carbonate.

Convergent evidence on the higher level of inorganic phosphate in zinc deficient stems was obtained from the reaction of free hand sections immersed in weak silver nitrate solution for a short time, followed by washing and immersion in sodium thiosulfate solution. After another washing, the sections were mounted in glycerine jelly. The contrast in appearance of the sections of healthy and of affected stems (fig. 3) after this treatment affirms the conclusions drawn from the preceding tests. The black material is interpreted as silver which had been precipitated by phosphate ions plus a certain amount of phenolic compounds, but the latter were comparatively scarce in these tissues. Inorganic phosphate was obviously more abundant in the cortex and pith of affected stems. The xylem in both stems contained phosphate which can be regarded as a solute in transit from the root to other centers of transformation. The heavier deposit in the cortex and pith of affected stems indicates that they had a higher level of phosphatase activity.

The black precipitate in parenchyma cells of affected stems was discernible principally in the periphery of the cell when highly magnified (fig. 4). This is interpreted to mean that in the affected cells phosphate esters had been largely converted into inorganic phosphate, thereby liberating energy for maintenance. Further evidence of damage to this cell is afforded by the aggregates of desolvated phenolic compounds and by the clumping of starch grains about the nucleus. In a healthy cell the inorganic phosphate would enter esters through a process of phosphorylation coupled with oxidation, but in a hypoplastic cell, where the necessary mechanism fails to work, the inorganic phosphate accumulates. Enzymic action had apparently liberated phosphate anions from phosphate esters and possibly from partially disintegrated cytoplasm.

To obtain further evidence on this point, I made tests for phosphatase by the method of Gomori (1939). Fresh sections of healthy and of zinc deficient stems were submerged in a solution which contained 2 per cent glycerophosphate and 2 per cent calcium nitrate where they were held at 30° to

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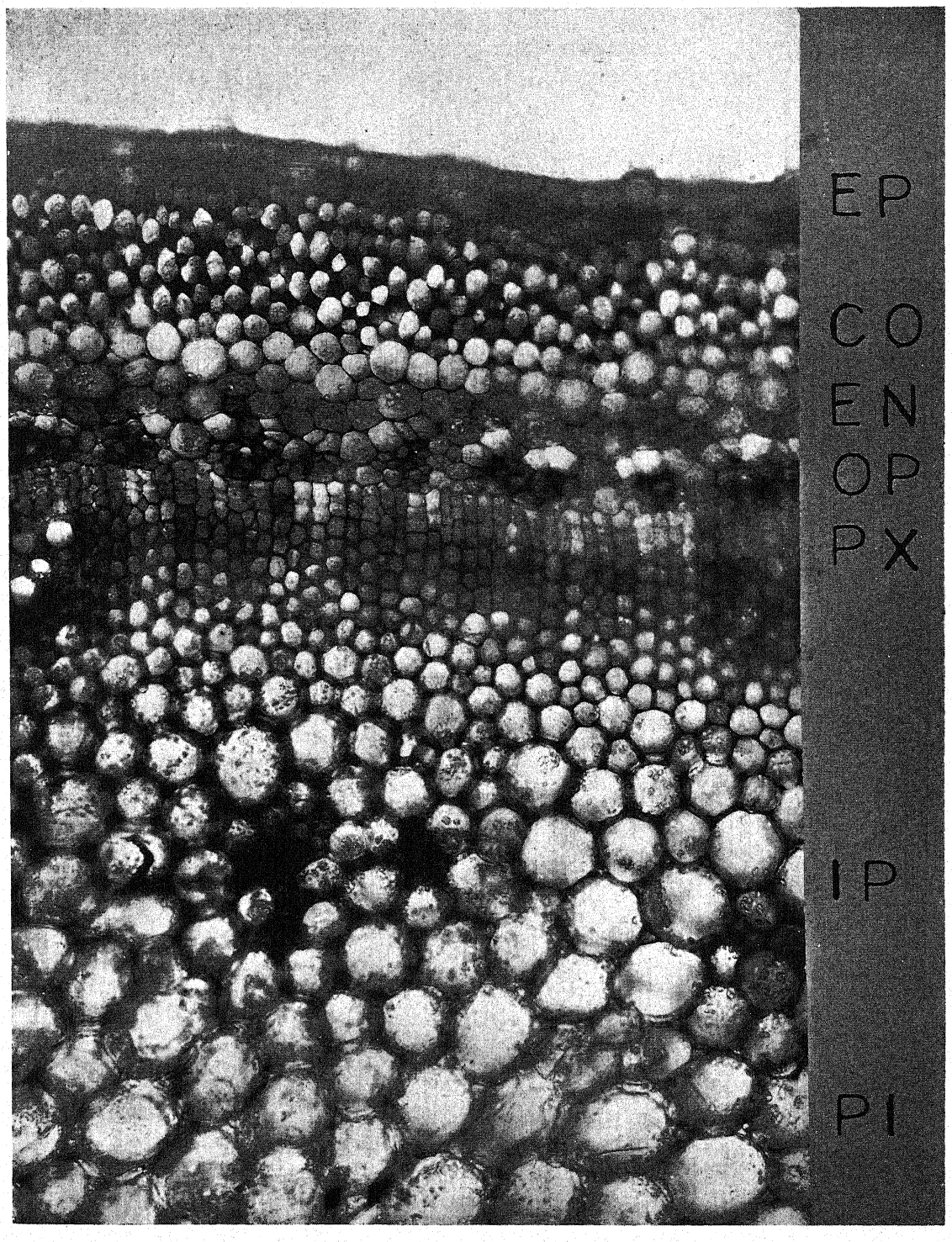


Fig. 1. Free hand section of the stem of a healthy tomato plant which had been supplied with 0.5 p.p.m. zinc. ×185. Inorganic phosphate appears black following application of the cobalt-ammonium sulfide test. EP, epidermis; CO, collenchyma; EN, endodermis; OP, outer phloem; PX, primary xylem; IP, inner phloem; PI, pith.

37°C. for 4 hours. The sections were next washed, placed in 2 per cent cobaltous nitrate solution, rewashed, and covered with a very dilute solution of ammonium sulfide whereupon the presence of inorganic phosphate was indicated by an intense black color. It is correct to infer that the phosphate demonstrated by this procedure came from the activity of an enzyme on glycerophosphate, because the 4-

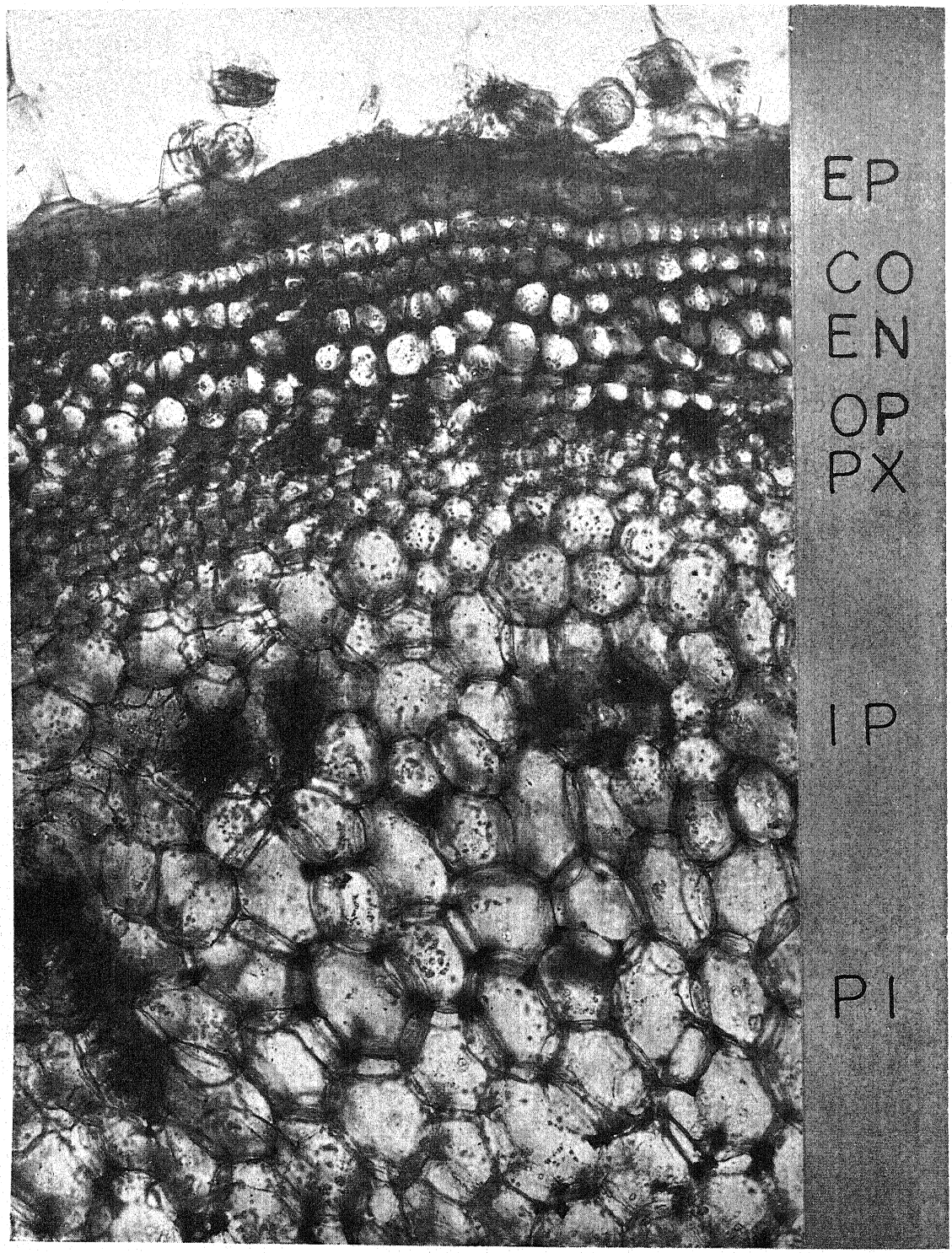


Fig. 2. Free hand section of the stem of an affected tomato plant which received no zinc. ×185. Inorganic phosphate appears black, following application of the cobalt-ammonium sulfide test. Abbreviations as in fig. 1.

hour incubation in the above mentioned solution would allow most of the inorganic phosphate to diffuse from the cells. Sections of affected stems were much darker colored by this treatment than similar sections of healthy stems. The results therefore indicate that there was an enhanced phosphatase activity in zinc deficient stems and confirm the interpretation stated in the previous paragraph.

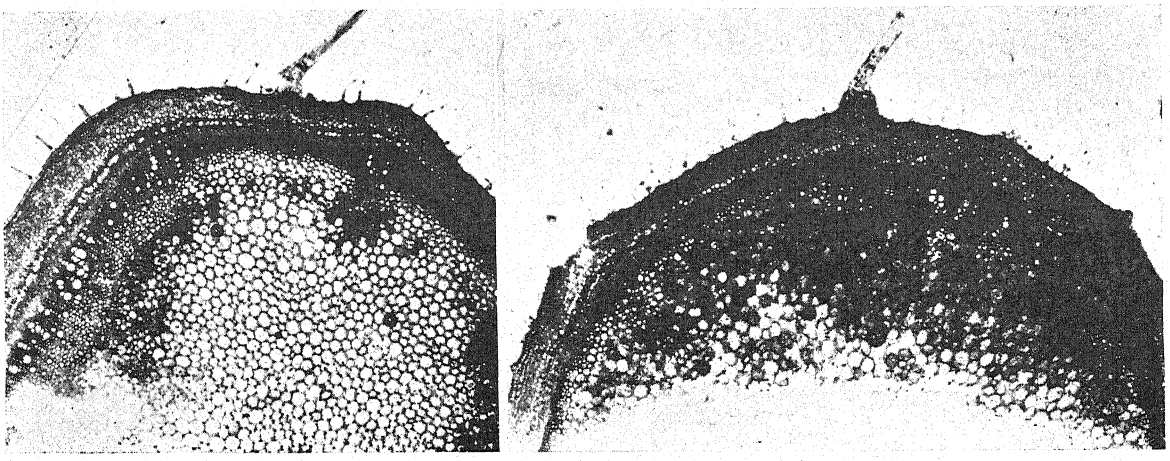


Fig. 3. Free hand sections of tomato stems. Left, healthy plant which grew in nutrient solution which had been supplied with 0.2 p.p.m. zinc. Right, affected plant which grew in a nutrient solution to which no zinc was added. Both sections had been treated with $AgNO_3$ and washed with sodium thiosulfate. ×16.

Additional information on the phosphate metabolism of mal-nourished plants was afforded by observations on tomato plants showing serious effects of boron deficiency. Fresh sections of affected stems also contained more diffusible phosphate than the healthy (tested by the molybdenic reagent). Pieces of stems of both classes of plants were fixed and dehydrated in 95 per cent alcohol to avoid precipitating phosphate compounds, embedded and sectioned with the microtome. When examined microscopically the results of other tests were clearly confirmed. The blue coloration of the thick-walled collenchyma cells, following the application of the molybdenic reagent showed that liberated phosphate ions were abundant and were adsorbed by the positive charges on the cell walls. The phloem elements contained amorphous material which also gave the blue molybdenic reaction; however, the pith parenchyma showed none. Concordant results were obtained from other studies on sections of healthy and boron deficient tomato stems which had been fixed in a solution of cobaltous nitrate plus formalin whereby phosphates were precipitated *in situ*.

Activity of oxidases.—Cytochemical tests of sections of living stems demonstrated that phenol oxidase is more abundant in zinc deficient than in healthy tomato stems. Sections which had been treated for a time in Nadi reagent in watch glasses, transferred to slides and examined under the microscope exhibited a beautiful zonation of colors. The epidermis, collenchyma, and xylem parenchyma were blue, in contrast to the unstained phloem, pitted and annular vessels and pith. In other words, oxidases were chiefly located in the extra-stelar tissues. Furthermore, the intenser color in zinc deficient material indicates clearly that such plants contained more phenol oxidase than stems of healthy plants, which had grown in nutrient solutions containing 0.5 p.p.m. zinc.

The oxidases could function smoothly as long as they were in contact with substances which could donate hydrogen. The most obvious source of such substances would be the leaves, but since the leaves of zinc deficient tomato plants are notably affected in form and function, the supply of necessary substrates would be seriously restricted. The result would be that many of the components of the cell would be irreversibly oxidized.

The low level of hydrogenases in the cells of affected tomato plants was revealed by tests on the power of fresh sections to reduce methylene blue when they were laid in drops of methylene blue in hollow ground glass slides and covered with slips whose edges were sealed with vaseline. Sections of zinc deficient plants were, generally, more deeply stained by methylene blue than those of the healthy, indicating less hydrogenase and more oxidase. In

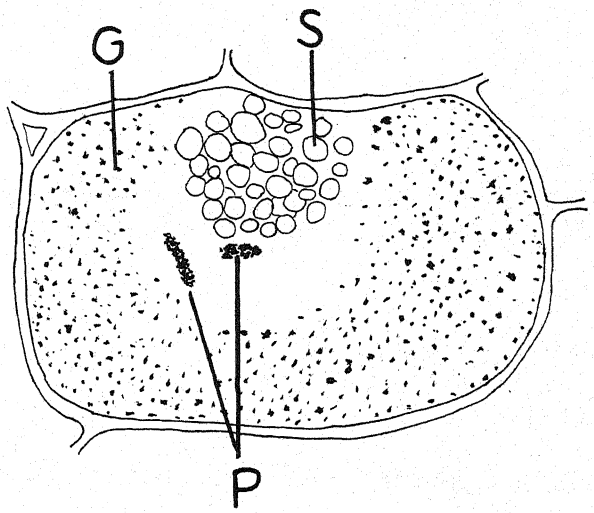


Fig. 4. Parenchyma cell from the pith of the third internode of a zinc deficient tomato plant. Drawn from a fresh section treated with $AgNO_3$. G, granules of inorganic phosphate; P, aggregates of phenolic material; S, starch grains clustered about the nucleus.

the former the epidermis, collenchyma, secondary xylem and pith were strongly stained; the cambium was faintly blue, whereas the endodermis, bast fibers and medullary rays were unstained. In the other lot of sections (healthy), where there appeared to be more hydrogenase activity, the zonation was substantially the same as above mentioned, except that the cambium was greenish instead of pale blue and the methylene blue was, in general, more rapidly reduced in equal periods of time.

The leaf petioles of tomato plants were examined because it is known that they also show characteristic external symptoms of zinc deficiency. Application of the Gomori test to fresh transverse sections of petioles showed that the cortical parenchyma of the healthy contained a very small amount of inorganic phosphate in marked contrast to the affected petioles of zinc deficient plants. In sections of the latter, black granules of cobalt sulfide were abundant, especially in the periphery of the cells, and in the majority of cases showed no Brownian movement, in contrast to the movement of calcium oxalate crystals in the cell vacuoles. The stelar tissue showed differences of the same kind but less in degree. The observations were therefore in harmony with those previously recorded for stems in showing that the extra-stelar cells of zinc deficient plants are the site of enhanced phosphatase activity.

It is appropriate to remark at this point that Vogeler, Umbreit, *et al.* (1942) have revealed a new and striking example of the general occurrence in nature of a linkage between oxidation and phosphate turnover. The oxidative phase was found to be accompanied by phosphate fixation, and the reductive phase of carbon dioxide fixation by a release of the phosphate. This fact and the oxidation of sulfhydryl compounds will be discussed in a subsequent paragraph.

Leaflets.—The dwarfed, chlorotic, involuted condition of the leaflets on zinc deficient plants has been mentioned above. It remains to discuss the cytological features of the healthy and affected leaflets. The extent to which hypoplasia and degeneration are associated with deficiency of zinc has been described in previous papers (Reed, 1938, 1939). Repetition is not necessary, but other effects will be described in succeeding paragraphs.

Slides bearing sections which had been fixed in Nemec's solution were held for 2 or 3 days in molybdenic reagent whereby bound phosphorus in plastids and nuclei of the parenchyma cells was stained blue. Starch was present in the plastids, but was not abundant. The phloem was not stained, indicating minimum disorganization. When stained with iron-alum-haematoxylin the picture was similar to that just described, though starch grains were not so readily seen, and the plastids were thin ovoids lying close to the parietal walls of the cells. Other sections of the same leaflets stained with safranin and fast green exhibited more seriously affected palisade cells whose plastids were weakly stained or had even vanished as a result of the lytic action of some unknown factor.

The palisade parenchyma cells of affected leaflets characteristically contained oxidized phenolic compounds in two forms: (a) globules which had been stained by the chromates of the fixing fluid, often containing cores of darker, denser material unstained by safranin, and (b) complex coacervates, each containing a central core which was stained an old rose color and surrounded by two distinct layers—an inner unstained, and an outer deeply stained. In healthy cells, where the respiration is compensated, the phenolic compounds are reduced back to the dihydroxy form as fast as they are oxidized to quinones. Therefore they retain their original physical as well as chemical properties. In affected cells the respiration may be non-compensated, however, in which case the phenolic compounds, having been oxidized to quinones, do not revert to their original condition because of the lack of activated hydrogen. As a matter of fact, they become subject to further oxidations resulting in concomitant changes in their physical condition. Molecules of polyphenols, originally dispersed at random in the vacuolar solution, become aggregated into larger groups of polymers which become more densely clustered at certain loci (Reed and Dufrenoy, 1942). The formation of a layer of phospholipid on the surface of the coacervates removes from the vacuolar solution one of the most essential substrates.

The palisade parenchyma cells of affected leaflets are also hypertrophied. The factors responsible for this increased size have not been determined. They either inhibit nuclear divisions through a stoppage of the partition of the chromatin bodies, or greatly promote cell enlargement. It has been known for a long time that nuclear divisions do not often occur in cells when the rate of metabolism is high, or when growth in volume is proceeding at a maximum rate. The latter seems applicable to the cells of these leaves.

The relative abundance of phosphates in healthy and affected leaflets was investigated by the application of the Gomori test to sections of fresh mate-

Table 1. *Carbohydrates in healthy and zinc deficient tomato leaves.*

Leaves	Per cent dry weight	Per cent of carbohydrates on dry weight basis: Reducing sugars	Sucrose	Starch	Starch (colorimetric)
Healthy (0.5 p.p.m. zinc)	11.8	3.75	2.00	1.50	1.59
Affected (no zinc)	13.9	4.17	1.75	.70	.75

rial. The black granules indicative of phosphate were not discernible in sections of healthy leaflets from plants which had grown in nutrient solutions containing 0.1 or 0.5 p.p.m. zinc. However, black granules were relatively abundant in the assimilative tissue of leaflets of plants which had been supplied with less than 0.1 p.p.m. zinc. The granules had a pronounced tendency to accumulate at one end of the cell, suggesting that there were intracellular gradients of phosphatase activity. Other sections of fresh material treated with the molybdenic reagent developed a strong blue color which diffused into the solution indicating, in harmony with previous observations, that the phosphates in the affected leaflets were water-soluble. The reagent also revealed coacervates in the parenchyma cells of affected leaflets. Similar cells of healthy leaflets showed a minimum of free phosphate and no coacervates.

Dilute neutral red solution penetrated slowly into the hypoplastic cells of the affected leaflets, indicating that they had a low pH; eventually it penetrated the vacuoles and revealed the presence of coacervates in the majority of cells. Its action on the chloroplasts confirmed foregoing statements.

Carbohydrates.—Available cytological technique does not afford information concerning the sugar in plant leaves, yet the investigation would be incomplete without obtaining some idea of the relative amounts of sugars and starches in the healthy and zinc deficient leaflets of tomato plants. Mr. Joseph Katz kindly made analyses of leaves harvested in September 1945 from plants in one of my experiments (table 1). The colorimetric determination of starch shown in the last column was a comparison of the starch content of fresh tomato leaves with a standard potato starch suspension, however, the figures given in the table are expressed on a dry weight basis.

It is apparent from the data that the leaves of zinc deficient plants contained more reducing sugar, but slightly less sucrose and starch than those of healthy plants. The results are in harmony with, and extend the findings derived from investigations of zinc deficient orange leaves ("mottle leaf"). Haas (1932) found that the sugar of mottle leaf orange cuttings previously dipped in a zinc-lime-water mixture disappeared more rapidly than that of untreated cuttings grown under similar conditions. It is well known that the plant obtains a large part of the necessary energy from the oxidation of sugars. Not all the energy obtained from this oxidation is used for growth. A portion, which we might term the "overhead" is used for the maintenance of the plant organization.

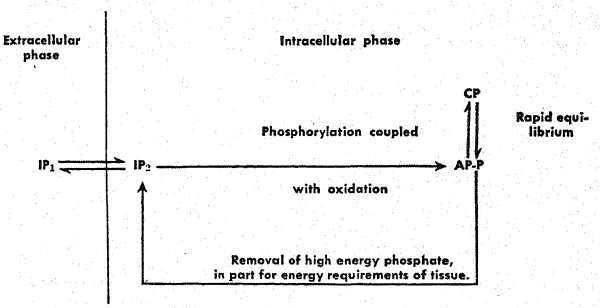
It is now possible to discuss the carbohydrate transformations in the light of evidence on the upset condition of phosphate esters. The leaves of zinc deficient plants contained more reducing sugar on the dry weight basis. The question arises, "Why did the plant not use that sugar for growth or for maintenance?" Since glucose and phosphate accumulated, it is plausible to assume that the stoppage is due to the lack of an enzyme like hexokinase which can catalyze the formation of glucose-6-phosphate according to the scheme:

$$PO_4 + \text{glucose} + ATP \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + ADP.$$

The problem is admittedly complex and additional investigations are needed to elucidate the relations of phosphates to carbohydrate transformations in these leaves. It can be said that the adsorption of phospholipids on the surfaces of coacervates by a molecular orientation at the interphases ties up an important link in the chain of metabolic activities which are concerned with carbohydrate transformations.

Discussion.—It cannot be gainsaid that zinc plays an important role in the physiology of plants as well as animals and that it enters intimately into complexes with various important cell constituents. Like iron and copper, it probably forms compounds with various proteins which have specific properties and functions. It is now in order to assess the results above reported and to link them to some of the basic processes in cell metabolism.

The data herewith presented make it possible to visualize certain aspects of phosphate turnover in the plant in relation to zinc and possibly other essential elements. The role of phosphate in carbohydrate transformations has been greatly illumined since the demonstration by Harden and Young (1906) that it has an important function in carbohydrate fermentation. Most of the intracellular phosphate in normal cells is in organic combination where it is linked by energy-rich bonds. The water-soluble esters, especially phosphocreatine, the adenosine nucleotides and the sugar phosphates are items of importance in this connection. It evokes no surprise to find that in abnormal cells there is a higher level of inorganic phosphate. Phosphorylations are coupled with the oxidative steps in the combustion of carbohydrates and each phosphorylation goes through adenosine triphosphate, according to Furchgott and Schorr (1943). They represent the phenomena as follows:



IP_1, IP_2 = inorganic phosphate; AP–P = adenyl pyrophosphate; CP = creatine phosphate.

The processes of phosphorylation in plant cells may differ somewhat from this scheme, but it is

tremendously important to realize that the phosphorylated compounds are reservoirs of energy in the form of phosphate bond energy which may be released, thereby affording energy for the maintenance of essential cell processes.

Sulfhydril compounds, such as cystein, are present in all living cells and, being labile, exert an important effect on living matter. Evidence of the relation of zinc to the stability of those compounds in cells was obtained from the study of orange leaves affected by zinc deficiency (Reed and Dufrenoy, 1935). By a combination of microincineration and microanalysis it was demonstrated that zinc predominated in centers of high metabolic activity in healthy leaves, but was at a minimum in affected leaves. Warburg and Christian (1943) subsequently advanced our knowledge of the subject by their investigation of the biochemistry of the zymohexase of yeast. They conceived that the cleavage of sugar which may be represented thus,

$$1 \text{ mol hexosediphosphate} \rightleftarrows \begin{matrix} 1 \text{ mol aldotriosephosphate} \\ + \\ 1 \text{ mol ketotriosephosphate} \end{matrix}$$

is due to the action of zymohexase, a metal protein containing 1 gram atom Cu and 1 gram atom Zn per 3×10^6 grams protein whose activity may be blocked by the formation of a complex with cystein. The blocked enzyme may, however, be reactivated by certain metals, *e.g.*, zinc, copper, iron and cobalt. Of the last three mentioned metals it seems that only the "ous" is effective in the reactivation, when the enzyme is protected from being oxidized by oxygen (as in argon). Zinc reactivates it, however, regardless of the oxygen tension and its presence is therefore of prime importance in the sugar metabolism of the cell. Warburg and Christian furthermore concluded that neutral cystein may bring about an equilibrium between free and bound zinc by binding the former to sulfhydril as defined by the formula

$$\frac{\text{zinc} \times (\text{cystein})^n}{\text{zinc complex}} = K$$

implying that the dissociable zinc-cystein complex plays a role in regulating the ionic concentration of zinc similar to that of weakly dissociable acids or their salts in the regulation of H-ion concentration. Further researches must be made before it will be possible to say whether similar relations exist in a complex organism like a tomato plant, but the evidence thus far obtained lends support to the idea that there was in the dwarfed, chlorotic plants some block in the enzyme system which controlled a fundamentally important process in the utilization of sugars. The data now at hand are sufficient, however, to justify the conclusion that the presence of an adequate supply of zinc in an active plant cell is necessary for the production and consumption of carbohydrates.

SUMMARY

Stems and leaves of tomato plants affected by zinc deficiency are dwarfed, pale green and more or less necrotic. The development of the meristematic cells of the stem is inhibited. The parenchyma cells of the leaf are hypertrophied and eventually their protoplasmic architecture is destroyed.

Cytochemical tests demonstrated that the extrastelar region and the phloem of the stems of affected plants contained relatively greater amounts of inorganic phosphate and of phenol oxidase than the healthy. They also showed that affected stems were richer in phosphatase and poorer in hydrogenase.

Convergent lines of evidence make it clear that the phosphate turnover in zinc deficient plants is so altered that cellular activities are profoundly disrupted.

The palisade parenchyma cells of leaflets of zinc deficient plants had a low pH and coacervates had been formed in the majority of them. Chloroplasts were relatively few.

The leaves of affected plants contained more reducing sugars, but slightly less sucrose and starch than those of healthy plants, indicating that one of the essential enzyme systems failed or was blocked in the mal-nourished plants.

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POLYPLOID MITOSIS AS A NORMALLY OCCURRING FACTOR IN THE DEVELOPMENT OF ALLIUM CEPA L.[1]

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THE IDEA that polyploidy is one of the causal factors involved in the normal development of many plants and animals is supported by an ever increasing accumulation of evidence. In the case of animals a good reference list of the literature may be found in the paper of Mickey (1945). Levan (1944) gave a brief review of the literature on this phenomenon as found in plants. The first suggestion that polyploidy might be a factor in the normal ontogeny of diploid plants was made by Winkler (1916) in his classical paper. He found polyploid mitotic divisions in the large cells of certain tissues of mature tomato plants. Several investigators (for literature see Berger, 1941) have described the very precise formation of polyploid cells termed polysomaty found in the root tips of spinach and a few other plants.

Most of the evidence for polyploid tissues has, however, been of an indirect nature involving secondary criteria such as cell size or number of heteropycnotic chromosomes (Geitler, 1939), or has dealt with material treated with growth promoting substances (Dermen, 1941) or stimulated by wounding or by bacterial infection (Wipf and Cooper, 1940). In other instances the polyploid condition was found only in a certain percentage of the plants investigated leaving in doubt the question of the normality and constancy of the phenomenon (Levan, 1944). Cases of direct evidence from polyploid mitoses are rare. A newly found case of this type is here described.

OBSERVATIONS.—While studying the cytological features accompanying development in *Allium cepa* it was found that all young seedlings of this species regularly formed a certain number of tetraploid cells which underwent normal mitosis. In young seedlings ranging from 10 to 40 mm. in length these 4n divisions were found in the mesophyll cells of the cotyledon (fig. 1 and 2) and in the cortex cells of the transition region between root and shoot (fig. 3 and 4). No polyploid divisions were ever found in the untreated root (fig. 5). In the cotyledon the tetraploid divisions were more abundant in the basal portion where they were found near the epidermis and endodermis as well as in the intermediate region. In the region of transition between root and shoot 4n divisions were likewise found to be distributed at random throughout the cortex. Apparently they were not restricted to the vicinity

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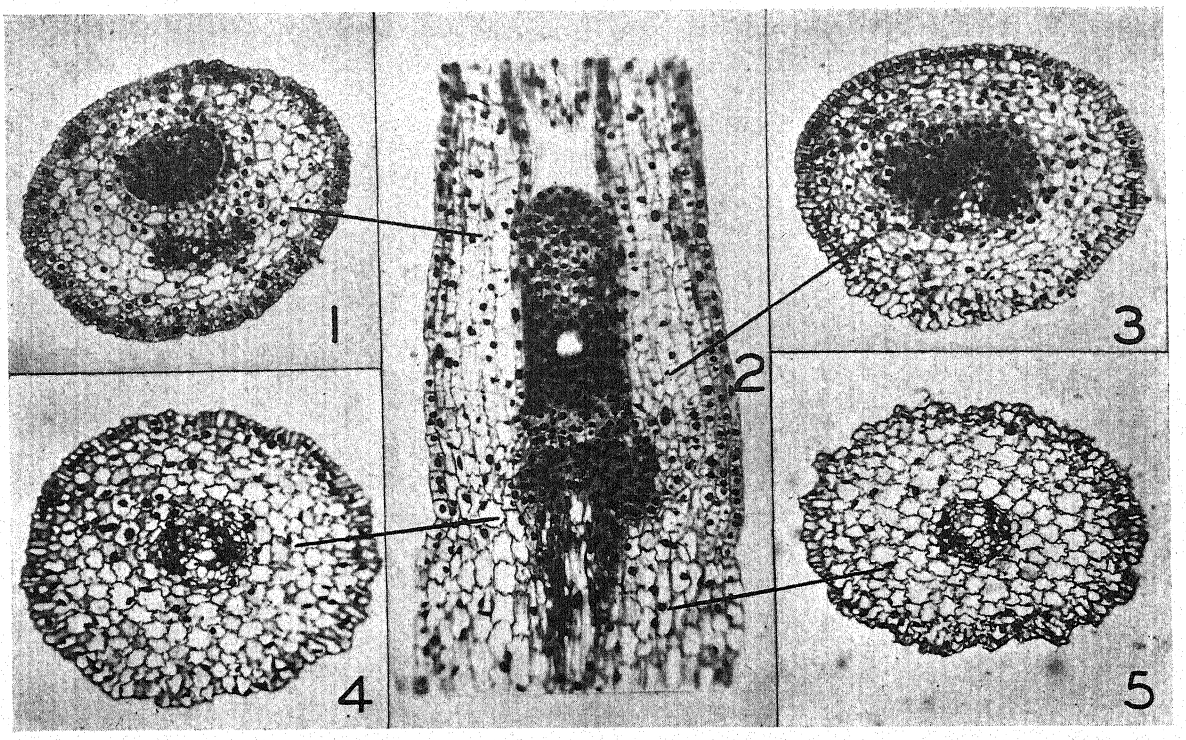
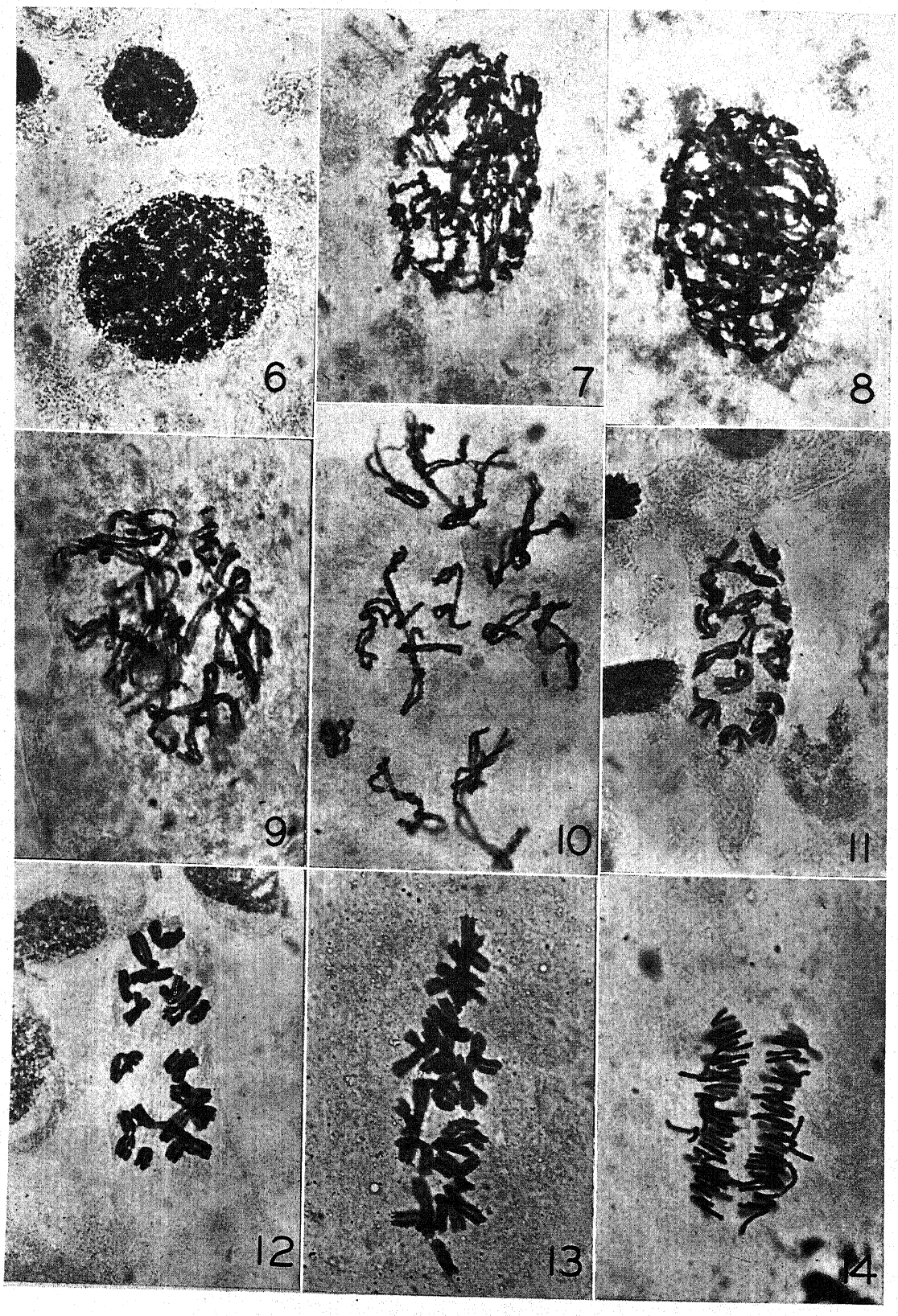


Fig. 1–5. Photomicrographs of cross and longitudinal sections of young seedlings of *Allium cepa*. ×75.—Fig. 1. Section through cotyledon and first leaf.—Fig. 2. Median longitudinal section of region of transition between root and shoot.—Fig. 3 and 4. Sections through region of transition.—Fig. 5. Section through root.


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of root primordia. No octoploid divisions were found. In seedlings more than 40 mm. in length few divisions of any kind were found in the cortex but in the meristematic regions of both root and shoot diploid divisions were abundant.

Cytological characteristics of 4n divisions.—Tetraploid divisions were found in large cells only (fig. 6). From earliest observable prophase to metaphase the chromosomes in such cells were found to be closely paired and relationally coiled (fig. 7, 8, and 9) indicating that the cells had not undergone mitosis since becoming tetraploid. Although many hundred seedlings were smeared and examined cytologically no instance of a 4n prophase with unpaired chromosomes was observed. In view of the ease with which paired and unpaired prophases can be distinguished in *Spinacia* where they are known to occur (Berger, 1941), the absence of unpaired prophases in *Allium* was taken to indicate that these tetraploid cells undergo at most only one mitosis as tetraploids. The spindle attachment (SA-region) of the paired and relationally coiled chromosomes remained single and undivided until prometaphase or metaphase (fig. 10, 11, and 12). At prometaphase such cells showed the diploid number (16) of tetrachromosomes, *i.e.*, two chromosomes united at a single SA-region and relationally coiled. The two chromatids of each chromosome were also relationally coiled. Two successive divisions of the SA-regions occurred at metaphase, the first resulted in 32 closely paired metaphase chromosomes (fig. 13) and the second was followed by normal anaphase separation (fig. 14).

Origin of the tetraploid condition.—The closely paired and relationally coiled condition of the chromosomes in very early prophase together with the undivided condition of their SA-regions is clear evidence that they have not separated since the time of their reproduction. The polyploid condition must therefore have arisen in the preceding resting stage by a process of double reproduction of chromosomes as described for *Spinacia* by Gentcheff and Gustafsson (1939) and Berger (1941). This process cannot properly be termed endomitosis since no endoprophase, endometaphase, endoanaphase or endotelophase stages as described by Geitler (1939) are involved.

Binucleate cells and mitotic aberrations.—In the same regions in which the tetraploid divisions were found binucleate cells and various mitotic aberrations were of common occurrence. The abnormalities included anaphase bridges, chromosomal fragments and resting cells with one or more micronuclei. It is probable that these aberrations are in no way connected with the tetraploid cells and divisions previously described. Nichols (1941) and unpublished work of the author clearly showed that mitotic aberrations such as anaphase bridges and chromosomal fragments are regularly found in root tips from seedlings of *Allium*. Such cells and their descendants are probably eliminated as development proceeds and while they furnish a satisfactory explanation of the binucleate cells found they are incapable of explaining the structure of tetrachromosomes or the pairing and relational coiling always found in tetraploid divisions. Occasionally dumb-bell shaped nuclei were found which apparently resulted from unbroken anaphase bridges. Even more rarely cells with nuclei of this type were found in prophase. If mitosis was completed in such cells tetraploid metaphases with unpaired chromosomes should be formed yet this condition was never found in the cells of any of several hundred plants studied.

SUMMARY

In seedlings of *Allium cepa* some tetraploid cells are formed and undergo one mitotic division during normal development. The tetraploid cells are found in the mesophyll of the cotyledon and in the cortex of the transition region between root and shoot. They are not found in the root. The tetraploid condition results from a double chromosome reduplication in the resting stage.

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Fig. 6–14. Photomicrographs of mitotic stages in tetraploid cells. ×750. All figures are of aceto-orcein smear preparations.—Fig. 6. Resting stages, 2n and 4n.—Fig. 7–10. Successive prophase stages showing paired and relationally coiled chromosomes.—Fig. 11–12. Prometaphase stages showing sixteen tetrachromosomes.—Fig. 13. Metaphase after first division of SA-regions showing thirty-two metaphase chromosomes.—Fig. 14. Anaphase, thirty-two chromosomes in each group.

PRODUCTION OF BIOCHEMICAL MUTATIONS IN PENICILLIUM [1]

David Bonner[2]

THE PRODUCTION of strains of *Neurospora crassa* characterized by a loss of biosynthetic ability is well known, and such strains have contributed materially to our knowledge of biosynthetic processes and their genetic control (see reviews, Beadle, 1945; Bonner, in press). During the course of attempts to induce mutations in *Penicillium* which might yield strains with higher penicillin yields, a number of strains have been obtained having nutritional requirements more complex than those of the parent strain. *Penicillium notatum* is an imperfect fungus, and it is therefore impossible to demonstrate the genetic basis of induced biochemical deficiencies. However, several of these strains are interesting from a biochemical standpoint, and probably many of them will prove useful in studies of biosynthesis and of comparative biochemistry. This paper deals with the methods of producing and detecting strains of *Penicillium* characterized by nutritional deficiencies, and records the strains that have been obtained.

STOCKS AND MEDIA.—Stocks of two species were used in this work, *P. notatum* and the closely related species *P. chrysogenum*. Table 1 lists the several strains used and their interrelationships. In all cases the various strains used in each species represent variants with regard to penicillin production, and all have the same nutritional requirements.

All parent strains used grow satisfactorily on a medium containing only salts, inorganic nitrogen, and a carbon source, such as glucose or sucrose. They have no accessory growth factor requirements. The basic, or minimal, medium used is Czapek-Dox medium containing the following ingredients in grams/liter: $NaNO_3$, 3.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; KCl, 0.5; $FeSO_4$, 0.1; and sucrose, 30.0. To insure growth of new strains having additional nutritional requirements, treated spores were allowed to germinate and grow on a medium supplemented with growth factors. The "complete" medium used is the penicillin sporulation medium of the Northern Regional Research Laboratory and contains the following ingredients in grams/liter: glycerine, 7.5; Brer Rabbit Molasses, 7.5; Bacto Peptone, 5.0; NaCl, 4.0; $MgSO_4 \cdot 7H_2O$, 0.05; KH_2PO_4, 0.06; ferric tartrate, 0.003; $CuSO_4$, 0.001; $CaCO_3$, 0.05. This medium is ordinarily solidified with 2 per cent agar for use as slants, and stock cultures are maintained on it. The vitamin solution contains the following ingredients in milligrams/liter of solution: thiamin, 100; riboflavin, 50; pyridoxin, 50; pantothenic acid, 200; p-aminobenzoic acid, 50; nicotinamide, 200; choline, 200; inositol, 400; alkali hydrolyzed yeast nucleic acid, 500; and folic acid equivalent to 4 micrograms pure substance. Casein hydrolysate is prepared by HCl hydrolysis and is made up to the equivalent of 50 milligrams casein per milliliter.

TABLE 1. *Penicillium strains used for irradiation. NRRL indicates Northern Regional Research Laboratory culture collection number.*

Strain number	Derived from strain	Derived by irradiation with
NRRL 1249·B21		
NRRL 832		
NRRL 1950		
NRRL 1951		
NRRL 1951·B25		
Stanford 622	NRRL 832	ultra violet
Stanford 4,171	NRRL 832	ultra violet
Stanford 23,248	NRRL 1951·B25	ultra violet
Stanford 25,038	NRRL 1951·B25	ultra violet
Stanford 25,099	NRRL 1951·B25	ultra violet
Stanford 35,217	Stanford 23,248	ultra violet
Stanford 35,347	Stanford 23,248	ultra violet
Stanford 45,417	Stanford 25,099	ultra violet
Minnesota X-1612	NRRL 1951·B25	X-ray

INDUCTION OF MUTATIONS.—Two types of treatment have been used to induce mutations, irradiation with ultraviolet radiation, and with x-rays.

Ultraviolet radiation treatments were made with a Westinghouse "Sterilamp" on conidia suspended in physiological salt solutions. This lamp emits more than 90 per cent of its energy at 2536 Å units, a wave length known to be highly effective in inducing mutations in other organisms. Suspensions of conidia in small Syracuse watch glasses were placed beneath the tube at a distance of two inches and treated 4 to 6 minutes, the suspensions being constantly agitated during irradiation. Approximately 100 ergs/mm^2 are delivered per second under these conditions, and these dosages give more than 95 per cent killing.

X-ray treatments were made by placing dry spores contained in small cellophane packets 6 cm. from the target of a Westinghouse "Duocondox" unit containing an industrial type oil cooled tube

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operated at 160 KV and 25 ma. No filter was used. Under these conditions the spores received irradiation at the rate of about 100 r units per second. Dosages of 50,000 to 200,000 r units were used, depending on the strain being irradiated. Marked variation in susceptibility to x-rays was observed between strains, strain X-1612 being much more sensitive than NRRL 1951·B25. The dosages used gave over 95 per cent killing.

As specific mutations were being sought, rather than data concerning mutation rate, great care was not exercised to operate under identical conditions each time a treatment was given.

Isolation of single strains.—After irradiation, spores are plated out on complete medium in shallow petri dishes at a concentration giving rise to separate colonies. Treated spores are suspended in sterile water with sodium·taurocholate added as a wetting agent. A serial dilution test is carried out by adding aliquots of the suspension to melted complete medium (allowed to cool to approximately 42°C.) and the medium poured into petri dishes. After 36 to 48 hours' incubation at 27°C. the correct dilution for the bulk of the suspension can be determined by inspection, and many new plates are then prepared at the correct dilution. Storage of the treated spore suspension for 36 to 48 hours in an ice chest does not give rise to harmful effects as far as can be determined. The treated spores are then incubated at 27°C. until the individual colonies are about 0.5 cm. in diameter. Under a low power dissecting microscope small bits of mycelia are then cut from the peripheries of single colonies and transferred to agar slants of complete medium. Peripheral sectors are taken to increase the probability of genetic homogeneity. If a spore is not uninucleate or if the chromosomes are already split at the time of treatment, selection of whole single colonies might give rise to genetically mixed cultures.

Detection of mutant strains.—The method of detecting biochemical mutations is essentially that employed by Beadle and Tatum (1945) in detecting biochemical mutations in *Neurospora*. Tubes of minimal medium are inoculated with spores from the isolation culture. The failure of a strain to grow on minimal medium indicates that it is unable to synthesize some factor present in the complete medium and lacking in the minimal medium. All strains unable to grow on minimal medium are retested in four stock solutions. The four stock solutions are minimal medium containing the following supplements: (1) 2 per cent yeast extract; (2) hydrolyzed casein (5 mg./cc.); (3) vitamin solution (2 ml. vitamin solution/100 ml. medium); (4) $(NH_4)_2SO_4$ substituted for $NaNO_3$ in the minimal medium. Growth in solutions 1 and 2 indicates that the strain is unable to make an amino acid or combination of amino acids; growth in 1 and 3 indicates that the strain is unable to synthesize a component of the vitamin solution; growth in 4 (probably growth in 1 and 2) indicates that the strain is unable to reduce nitrate nitrogen and must, therefore, receive a reduced form of nitrogen in the medium. Growth in solution 1 alone may indicate that the strain requires some heretofore unrecognized factor present in yeast extract. More often, however, it has been found to mean that the strain requires some known factor, but fails to grow on synthetic medium because of an inhibiting substance present in too high concentration (Bonner *et al.*, 1943; Doermann, 1944). From this preliminary screening the individual requirements are easily determined by appropriate testing of single substances or combinations of them.

Table 2. *Biochemical mutants of Penicillium notatum-chrysogenum.*

Accessory growth factor required	Number of strains derived	Accessory growth factor required	Number of strains derived
Biotin	5	Arginine	52
Choline	9	Arginine-Proline	13
Inositol	6	Cystine-Methionine	50
Nicotinic Acid	5	Histidine	16
p-Aminobenzoic Acid	6	Isoleucine	1
Pyridoxin	6	Leucine	12
Thiamin	18	Lysine	54
Yeast Nucleic Acid	19	Methionine	32
Nitrate Reducers	31	Phenylalanine	4
Unknowns	43	Proline	14
		Tryptophane	2

Results.—A total of 398 strains having deficiencies in synthetic ability were isolated from a total of 85,595 strains tested. Table 2 lists the types of mutations found and the number of mutant strains of each type isolated. The types of mutants and their frequencies are on the whole similar to those found in *Neurospora* (Beadle and Tatum, 1945). No mutations involving the production of riboflavin or pantothenic acid were observed in *Penicillium* analogous to those of *Neurospora*. On the other hand, a number of strains requiring the amino acid histidine were obtained in *Penicillium* but not in *Neurospora*. The mutation frequency is nearly the same as that found in *Neurospora* (Beadle and Tatum, 1945). Several attempts were made to induce heterocaryon formation as a test of genetic dissimilarity as described for *Neurospora* by Beadle and Coonradt (1944). Pontecorvo and Gemmell (1944) have observed heterocaryon formation between strains of *Penicillium* characterized by deficiencies in pigment production. Growth of a mixed culture of two different biochemical mutant strains when inoculated into a minimal medium, however, has not been observed.

Discussion.—Though it has been impossible to obtain direct proof of the genetic control of biochemical reactions in *Penicillium*, a comparison of the mutants found with those of *Neurospora* makes it almost certain that in *Penicillium* there is a genetic control of biochemical reactions similar to that found in *Neurospora*.

It has been possible in some instances to work out the series of reactions by which *Neurospora* synthesizes certain end products, and at the same time show the genetic control of each step in the synthesis (see review, Bonner, in press). The synthesis of arginine will best serve as illustration. Srb and Horowitz (1944) have shown that *Neurospora* synthesizes arginine by the series of reactions

$$X \xrightarrow{\text{genes } 4,5,6,7} \text{Ornithine} \xrightarrow{\text{genes } 2,3} \text{Citrulline} \xrightarrow{\text{gene } 1} \text{Arginine.}$$

Certain strains can use only arginine, others can use citrulline and arginine, and still others can use ornithine as well as citrulline and arginine. As only the three types are known, the order of these compounds is established as shown above. In each case, the mutant type differs from normal by a single gene.

The mutant strains of *Penicillium* requiring arginine may be classified in a similar manner and the series even extended. The argininеless strains of *Penicillium* may be classified according to their growth on arginine, citrulline, ornithine, proline and glutamic acid as shown in table 3.

The position of each of the compounds in an ordered series may be deduced from the fact that since all five strains can use arginine, and but four of these can use citrulline, then citrulline must act as a precursor of arginine. Similarly, ornithine is a precursor of citrulline. Proline, however, is not an immediate precursor of ornithine in the main chain of reactions leading to arginine synthesis, since strains are known which can use only proline. This would suggest that proline and ornithine have a common precursor and the reaction of precursor to proline is readily reversible. This observation is in agreement with the thesis of Shemin and Rittenberg (1945) of a close link between the synthesis of proline and arginine, but is not in agreement with their proposed cycle starting with ornithine. Following from previous arguments, glutamic acid is the precursor of the proposed intermediate. The series of reactions in the synthesis of proline and arginine in *Penicillium* might be represented as follows, with the genetic block at each step indicated by the strain number:

$$\text{Ornithine} \xrightarrow{6572} \text{Citrulline} \xrightarrow{3485} \text{Arginine.}$$

$$\text{Glutamic acid} \xrightarrow{24033} \text{Intermediate} \xrightarrow{9929} \text{Proline} \xrightarrow{6155}$$

The close similarity between these mutants and those known in *Neurospora* would argue for the existence of a similar control of biochemical reactions in both organisms.

A second case in which it has been possible to demonstrate the similarity of the *Penicillium* mutants to the *Neurospora* mutants is one recently reported by Tatum (1945). One might expect that a reaction blocked genetically would tend to accumulate the precursor immediately prior to the block. The accumulation of such a precursor could then be demonstrated by the growth of a second mutant type on the substance accumulated in which the genetic block occurs prior to that in the first type. Several such cases have been reported in *Neurospora* (Tatum *et al.*, 1944; Horowitz, 1946; Bonner and Beadle, 1946). Tatum (1945) has found that *Penicillium* strain 21464 can ultilize desthiobiotin as well as biotin. A second strain, 62078, needs added biotin but cannot utilize desthiobiotin. However, strain 62078 produces a substance which accumulates in the medium and which strain 21464 can use. From other evidence, this substance appears to be desthiobiotin.

From such evidence the mutant strains of *Penicillium* reported in this paper appear to represent true genetic modifications and suggest that in *Penicillium* there is a genetic control of biochemical reactions, as there is in *Neurospora*.

TABLE 3. *Argininеless strains of Penicillium.*

Strain number	Arginine	Citrulline	Ornithine	Proline	Glutamic acid
3485	+	—	—	—	—
6572	+	+	±	—	—
6155	+	+	+	—	—
9929	+	+	+	+	—
24033	+	+	+	+	+

SUMMARY

After ultraviolet radiation or irradiation with x-rays, 398 strains of *Penicillium notatum-chrysogenum* with altered nutritional requirements were isolated from a total of 85,595 strains tested. The means of obtaining and detecting such strains is given, as well as a summary of strains obtained. The biochemical relationship between proline, arginine and glutamic acid is indicated. Since *Penicillium notatum-chrysogenum* is an imperfect fungus, no genetic data have been obtained. Heterocaryon formation was not observed. Biochemical studies of certain strains suggest that these mutant types represent genetic modification and that in *Penicillium notatum-chrysogenum* there is a genetic control of biochemical reactions.

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THE DEVELOPMENT OF ENDOSPERM AND ANTIPODAL TISSUE IN ARGENTINE WAXY MAIZE [1]

John E. Sass

THE DEVELOPMENT of the endosperm of *Zea mays* has been studied in a series of investigations in which it was established that the endosperm is triploid, and that a residual mass of monoploid tissue, derived from the three antipodal cells, persists until maturity of the kernel. Randolph (1936) has reviewed the history of the subject, and has amplified our knowledge of the development of the caryopsis in many important details. Weatherwax (1926) figured the persistent antipodal tissue as a relatively small mass of abnormal cells. Randolph's figures show an appreciable mass of apparently normal, starch-filled cells, described as having been derived from proliferated antipodal cells, and enveloped in a layer that was designated aleurone.

The presence of a considerable amount of starch of entirely maternal origin would be of particular interest in a research program in which emphasis is placed on the relationship between the properties of starch and the genotype of the starch-bearing cells. Antipodal tissue may well contribute some starch. Ferguson (1927) has described an unusual situation in which diploid, maternal cells derived from the fused polar nuclei comprise the endosperm. Although Cooper (1946) is not in agreement with Ferguson's interpretation in Petunia, the possibility that "endosperm" tissue may originate from fused polar nuclei can not be excluded.

An opportunity to re-examine this relationship arose in connection with a study of "Argentine waxy" maize (Brimhall *et al.*, 1945). The starch extracted from this strain was found to contain 2 to 3 per cent amylose, the straight-chain starch which stains blue with iodine. In ordinary waxy maize, the starch in the triploid endosperm and in the monoploid pollen stains yellow-brown with iodine. A developmental study of the caryopsis of Argentine waxy maize was made to determine the location of this amylose "contaminant." Many of the sections of the kernels were found to have a large "island" of starch-filled cells near the style. Serial sections showed that these apparent islands are merely involuted lobes of the endosperm. The foregoing observations were made from kernels collected 20 days after pollination. It was obviously necessary to undertake further studies, beginning soon after pollination, to determine the origin of these lobes in relation to the development of the endosperm and antipodal tissues.

MATERIALS AND METHODS.—The kernels of Argentine waxy maize used in this study were obtained through the courtesy of Dr. G. F. Sprague, who made self-pollinations of field-grown plants in 1944 and 1945, and of greenhouse-grown plants during the intervening winter. Kernels were processed, sectioned and stained essentially as described recently (Sass, 1945). The most consistently satisfactory results were obtained by the use of chrome-acetic-formalin as a killing and storage fluid, a dioxan-normal butyl alcohol series for dehydration, and normal butyl alcohol with 5 per cent chloroform as the solvent for infiltration in paraffin. The low-magnification photomicrographs were taken with micro-tessar objectives, on a horizontal optical-bench type apparatus designed and built in this laboratory. This apparatus will be described in a paper now in preparation. The photomicrographs of higher magnification were taken with the conventional Kohler system, using Leitz apochromatic objectives, periplan oculars and well-corrected substage and lamp condensers.

OBSERVATIONS.—Three days after pollination, the endosperm in some kernels is still in the free-

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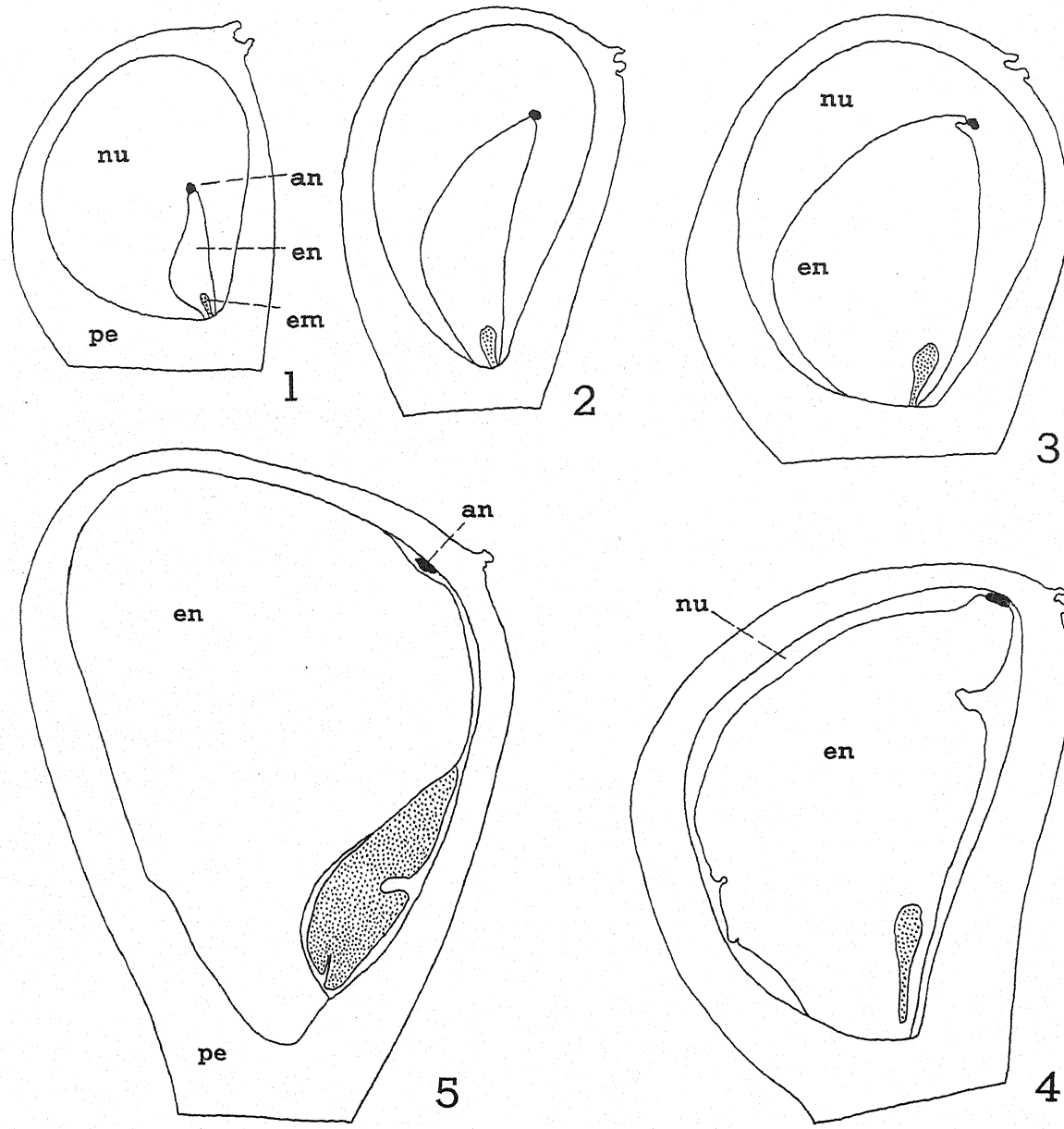


Fig. 1–5. Semi-diagrammatic projection drawings of sections of kernels, approximately 16×.—Fig. 1. Four days after pollination. Endosperm half way across nucellus.—Fig. 2. Six days. Endosperm approaches stylar end.—Fig. 3. Eight days. Lobe appears at distal end of endosperm.—Fig. 4. Ten days. Antipodal tissue pressed against pericarp.—Fig. 5. Thirteen days. Nucellus completely displaced by endosperm, antipodal tissue pressed against pericarp. Legend: al, aleurone; an, antipodal tissue; en, endosperm; nu, nucellus; pe, pericarp; em, embryo.

nuclear condition (fig. 6), whereas in other kernels the endosperm is partly or entirely cellular. In four to five days the endosperm becomes entirely cellular (fig. 7). The antipodal tissue at the distal end of the endosperm occupies a relatively small volume. By the fourth day the endosperm has grown through half of the length of the nucellus (fig. 1), and two-thirds of the length in six days (fig. 2), but the antipodal mass undergoes little or no enlargement. Continued enlargement of endosperm is not accompanied by appreciable enlargement of the antipodal mass, although a few abortive mitotic figures were observed in antipodal cells at eight days.

Between the eighth and tenth day, localized acceleration of cell division may occur in the distal region of the endosperm. This activity produces a projecting lobe, usually in contact with the small mass of antipodal tissue (fig. 3, 8, 9). Approximately 10 days after pollination the endosperm reaches the stylar end of the kernel. The nucellus has become greatly reduced and the antipodal cells

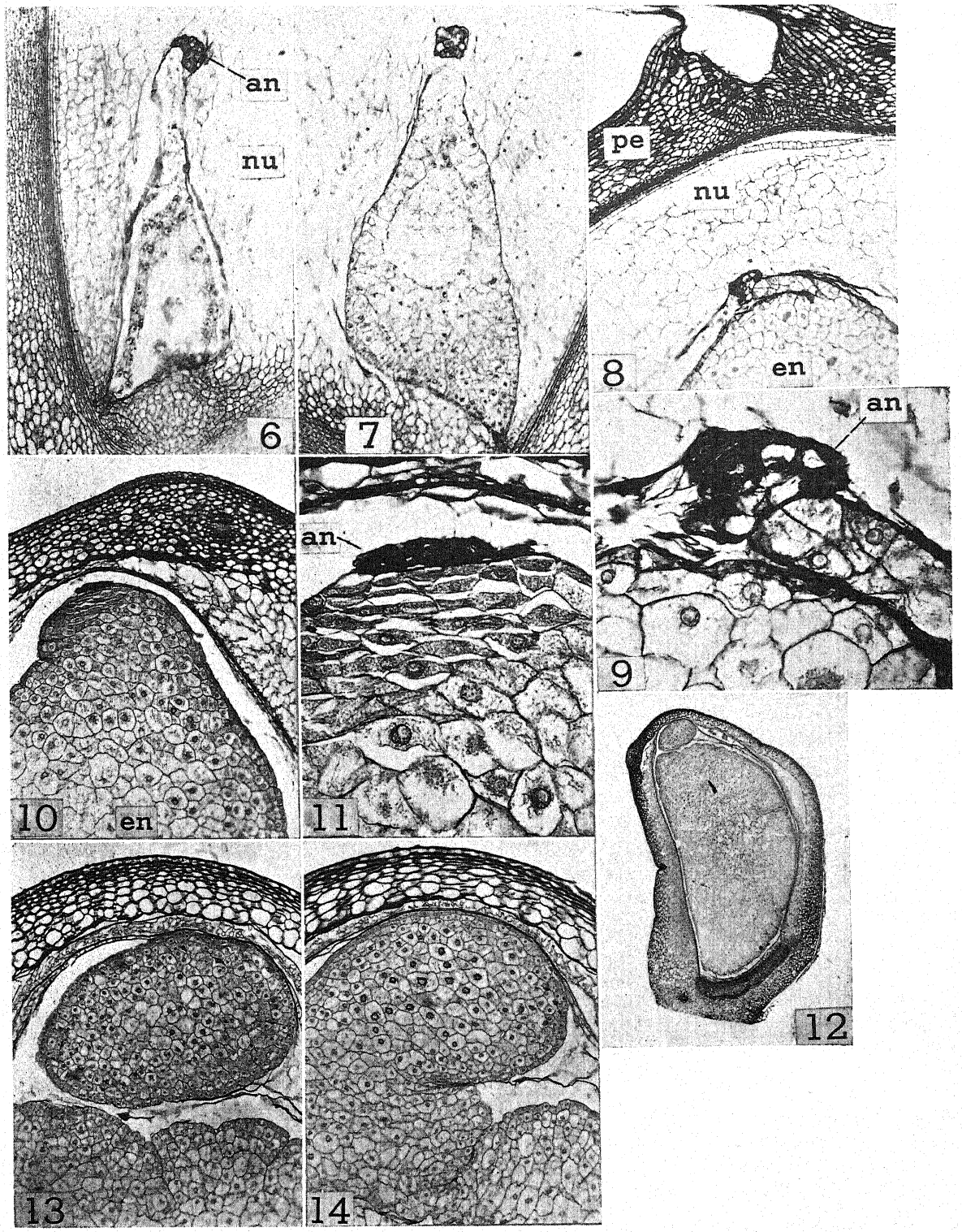


Fig. 6–14.—Fig. 6. Three days after pollination, antipodal tissue at distal end of free-nuclear endosperm. 66×.—Fig. 7. Four days. Antipodal tissue at distal end of cellular endosperm. 66×.—Fig. 8. Eight days. Lobe beginning to form at distal end of endosperm, in contact with antipodal cells. 66×.—Fig. 9. Enlarged view of endosperm lobe and antipodal tissue of figure 8. 280×.—Fig. 10. Ten days. Endosperm lobe and crushed antipodal tissue. 66×.—Fig. 11. Enlarged view of antipodal region of figure 10. 280×.—Fig. 12. Ten days. Apparent "island" of tissue at stylar end of endosperm. 10×.—Fig. 13. Enlarged view of "island" shown in fig. 12. 66×.—Fig. 14. Section of same kernel sectioned serially, showing continuity of endosperm. 66×.

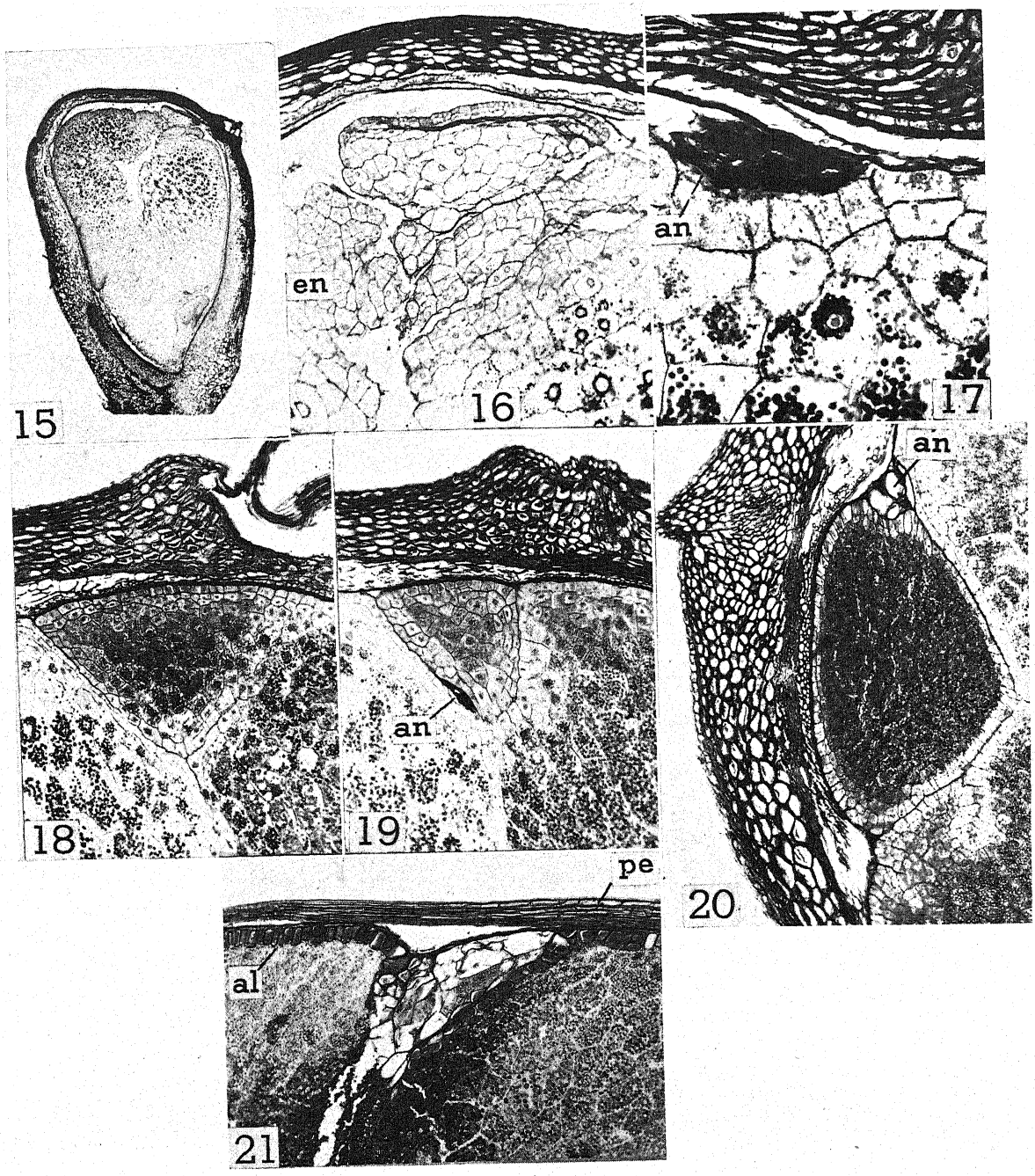


Fig. 15–21.—Fig. 15. Twelve days. Median section of kernel showing embryo and several distal lobes of the endosperm. 10×.—Fig. 16. Enlarged detail of endosperm lobes. 66×.—Fig. 17. Thirteen days. Antipodal cells crushed against stylar end of kernel. 280×.—Fig. 18–19. Sixteen days. Two sections from a serially sectioned kernel, showing continuity of endosperm, an apparent "island" of endosperm tissue, and a buried and crushed mass of antipodal cells. 66×.—Fig. 20. Twenty days. Section of endosperm lobe, showing disintegrated antipodal cells. 80×.—Fig. 21. Thirty-five days. Involution of endosperm. 66×.

show varying degrees of compression and disintegration (fig. 4, 10, 11). One or more lobes of the endosperm continue to enlarge, and in section give the appearance of detached islands of tissue (fig. 12, 13–16). Serial sections show clearly that these regions are continuous with the endosperm, and are merely lobes or involutions of endosperm tissue (fig. 13–16). The lobes frequently are not in the same median plane as the embryo. In such kernels the longitudinal sections that pass through the embryo do not show lobes or islands.

The space between the lobes may become closed

as early as the thirteenth day, however, the crease remains visible, and the distal surface of the endosperm remains more or less indented. The antipodal tissue is still recognizable, pressed against the pericarp (fig. 5, 17).

The aleurone becomes well defined by the sixteenth day and envelopes the main body of the endosperm, and also follows the involutions. Thus, a section of a lobe appears to be an island of tissue enveloped in an aleurone layer. As in the younger kernels, serial sections show that the apparent island is a lobe or involution, bordered by an involuted aleurone layer (fig. 18, 19). In some kernels the antipodal cells become buried in an endosperm crease (fig. 19), in other kernels the antipodal tissue becomes compressed against the pericarp (fig. 17).

Deposition of starch progresses rapidly between the tenth and twentieth day, and the distribution pattern is well established in 20 days. The starch is packed most densely at the stylar end, and the density decreases gradually toward the base of the kernel. This is the characteristic pattern in flint and popcorn. The involutions of the endosperm are well defined at 20 days, and the disintegrating antipodal tissue can be recognized in favorable sections (fig. 20).

The involutions are still evident at 35 days. Some involutions are entirely closed, but they can be recognized by the double layer of infolded aleurone. Other involutions are still open and contain groups of apparently normal starch-filled cells, as well as groups of compressed, disintegrated cells (fig. 21). At this stage, the antipodal cells cannot be readily distinguished because some of the disintegrated cells may be pinched-off endosperm cells, derived as shown in fig. 16.

In view of the demonstrated lack of extensive proliferation of antipodal cells, and the destructive compression of these cells, it is certain that no antipodal cells remain in active, normal condition in the mature kernel, and these cells contribute negligible volume and no starch-bearing cells to the food reserve.

Lobed endosperm has been noted in other lines of maize. The formation of the more extreme lobing in relation to the antipodal cells in some of these lines will be described in a subsequent report.

Kernel sections were stained with iodine as described by Sass (1945). The starch throughout the triploid endosperm, including the involutions, stains pale violet, whereas the starch in the diploid embryo of the same kernel stains blue-black. The endosperm starch in ordinary waxy maize stains yellow-brown, whereas the starch in the embryo stains blue-black.

The foregoing study indicates the need for similar study of distinctly diverse types of maize, as well as a re-examination of varieties used in earlier studies.

SUMMARY

The development of endosperm and antipodal tissues was studied in a waxy flint maize from Argentina. The enlarging endosperm invades the nucellus and pushes the relatively inactive mass of antipodal tissue toward the style. A few abnormal mitotic figures were observed in antipodal cells eight days after pollination. The disintegrating antipodal cells become compressed against the pericarp, or enveloped in a fold of the endosperm ten days after pollination.

The distal surface of the endosperm produces one or more lobes, which usually retain continuity with the main body of the endosperm and become filled with starch. Small masses of endosperm may become isolated by the involuted aleurone. Such islands accumulate relatively little starch and become compressed by the starch-filled endosperm. In this strain of maize the antipodal tissue becomes obliterated and is not a factor in the quantity or character of the millable starch.

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STOCK–SCION RELATIONSHIPS IN TOMATOES [1]

Katherine S. Wilson and Carl L. Withner, Jr.

ALTHOUGH THE results of reciprocal grafting have frequently been studied, many problems concerning the effect of stock on scion still appear to be controversial. The concept of vegetative hybridization held by certain Russian scientists has been re-emphasized by the recent translation into English (1946) of Lysenko's "Heredity and its Variability" which was first published in 1943.[2] In tomatoes, for example, his workers have found that such characters as fruit size or color, number of carpels, leaf shape, and habits of vegetative growth may be transmitted from stock to scion under prescribed experimental conditions using mature plants for stocks and very young plants as scions. However, the effects of reciprocal grafting using seedling tomato plants for both stock and scion had been reported by Avakian and Jastreb (1941). In these experiments varieties with contrasting fruit colors, size or shape were top-grafted after the plants had produced three to four leaves. Among changes described was the remarkable case of a graft between red "Mexican 353" (stock) and white-fruited "Albino" (scion). The plant produced a total of twenty-two fruits—eleven red, eight yellowish-white with pink stripes and dots, and three plain yellowish-white.

Less striking were the results of earlier investigations by Trowbridge (1880) and Heinicke (1927, 1936) who found that apples, when top-worked with other varieties, produced fruit below the graft unions displaying characters of the scions. Removing stock leaves and ringing the branches below the fruits helped intensify the scion effect. A chlorophyll-defective petunia was found by Wettstein and Pirschle (1938) to transmit leaf and shoot characters to normal plants by grafting. Pirschle (1939) made mineral analyses of the reciprocally grafted plants and found defective stocks had an inhibiting influence on the normal scions, while normal stocks had a beneficial effect on defective scions. Radium induced variations in *Antirrhinum* and tomato were transmitted from stock to scion according to Stein (1939). Many grafts were made by Daniel (1926) in an attempt to demonstrate reciprocal effects, and he thought many of these effects were inherited. Swarbrick (1930) and Tukey and Brase (1933) have thoroughly discussed the effects of various apple scions on their rootstocks and reviewed literature covering that subject.

Chemical differences between stock and scion

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[2] Since this has been in press, P. S. Hudson and R. H. Richens have published a scholarly review and critique of the work of Lysenko and his school. Our findings substantiate the views of these authors. "The New Genetics in the Soviet Union" is reviewed in Nature 158: 285–287, 1946, and in Science 104: 377–378, 1946.

TABLE 1. *Grafts between varieties differing in fruit characters and the number of plants to which each type of experimental treatment was applied. Bonny Best (BB), Crystal White (CW), Golden Queen (GQ), Red Plum (RP), and Yellow Pear (YP).*

Graft combinations				Fruit differences[a]				Experimental treatment[b]						
								Effects of stock on scion				Effects of scion on stock		
Scion	Stock	Number grafted	Number survived	Color	Shape	Size	Number grafted controls	(a)	(b)	(c)	(d)	(e)	(f)	(g)
BB	CW	12	8	×	..	..	1	..	1	1	..	2	2	1
CW	BB	12	5	×	..	..	1	1	1	..	..	..	1	1
GQ	BB	12	12	×	..	..	1	2	4	1	..	2	1	1
BB	GQ	12	11	×	..	..	1	..	1	..	1	3	3	2
RP	YP	12	8	×	×	..	2	2	3	1	..	..	..	..
YP	RP	12	9	×	×	..	2	2	2	1	1	..	1	..
YP	GQ	12	10	..	×	×	2	2	2	1	1	2	..	..
GQ	YP	12	8	..	×	×	1	1	2	1	..	2	..	1
RP	BB	12	9	..	×	×	2	..	..	1	2	..	3	1
BB	RP	12	12	..	×	×	2	1	2	1	1	2	2	1
YP	BB	12	12	×	×	×	1	1	2	1	1	2	2	2
BB	YP	12	9	×	×	×	2	..	3	2	..	2	..	..
CW	RP	12	4	×	×	×	1	..	1	1	..	..	..	1
RP	CW	12	9	×	×	×	2	..	..	..	1	2	2	2
GQ	RP	12	9	×	×	×	1	1	..	..	4	2	1	..
RP	GQ	12	11	×	×	×	2	2	2	1	2	..	1	1
YP	CW	12	8	×	×	×	1	..	2	..	..	2	2	1
CW	YP	12	6	×	×	×	1	1	2	1	..	..	..	1
Totals		216	160	14	14	12	26	16	30	14	14	23	21	16

[a] "×" indicates a difference in this character between the two varieties.

[b] Types of treatment are explained in table 2.



Fig. 1–4.—Fig. 1–3. Demonstration of grafting procedure.—Fig. 4. Typical grafted control plant bearing normal fruit of both varieties (Golden Queen—stock; Red Plum—scion). String label at base of scion stem indicates point of graft union.

have also been investigated. Rao and Berry (1940) studied carbohydrate relations between grafted varieties of apples. Hofmann (1927) found no transfer of anthocyanin from Refugee bean scions to Navy bean stalks, and Daniel (1926) found no transfer of inulin from artichoke scions to sunflower stocks.

The transmission of chemical substances across the graft union has been demonstrated in only a limited number of cases. Krajevoj and Nechaev (1941) found atropine was transferred from *Datura stramonium* rootstocks to *Solanum lycopersicum* (*Lycopersicum esculentum*) scions, and Dawson (1942) has shown that nicotine produced in tobacco roots is transported across the graft union into tomato scions.

Since results of the Russian workers were so striking and so much at variance with the reports of others, it has seemed worth while to the present writers to repeat, in so far as possible, the reciprocal grafting experiments with tomatoes by Avakian and Jastreb and to extend them by attempting to find physiological differences between the grafted and ungrafted plants as indicated by their content of certain major B vitamins. On the basis of Dawson's work it seemed logical to assume there might be differences in the ability of the organs of the different tomato varieties to synthesize or store these vitamins, and that these differences, if any, might be transmitted to stocks or scions.

Procedure.—*Grafting.*—Tomato plants used in this study consisted of five varieties which provided differences in color, shape and size of fruit. These were: Bonny Best (large red), Golden Queen (large yellow), Crystal White (large white), Red Plum (small red plum-shaped), and Yellow Pear (small yellow pear-shaped). Seeds were obtained from a commercial house and sown in flats in the greenhouse during the middle of March. Seedlings were transplanted into individual wooden containers, and when the plants had produced from three to five leaves, reciprocal top grafts were made in the manner described by Avakian and Jastreb. The tip, including the apex and several very young leaves, was removed with an oblique cut and replaced by the tip of another variety. The small scion was then held in place by binding the stem with a narrow strip of parafilm (fig. 1, 2, 3). Immediately after grafting, the plants were placed in the humid atmosphere of a wardian case where they were grown for a week or until the graft union had begun to heal. The parafilm was then removed, and the plants were placed in a shaded section of the greenhouse until the union had healed completely.

Grafts were made reciprocally between varieties differing in fruit size, shape, or color (table 1).

Most of the grafted plants were trained to two shoots, one from the stock and one from the scion, but some were limited to one scion shoot. Lateral branch production was prevented by regular and frequent removal of buds. Flower buds were also removed until June, when the plants were moved into the field for experimental treatment. During this transplantation care was taken to keep the graft sufficiently far above the soil level so that the production of roots above the graft union was prevented.

Various treatments were employed in order to provide maximum opportunity for the production of effects between stock and scion (table 2). These consisted of restriction of the number of shoots, restriction of fruit production to either stock or scion, and reduction of leaf area by defoliation of stock or scion after fruit had set. Shoots marked for defoliation were stripped of all leaves except two or three protecting the shoot apex, and were maintained in this state by removal of older tip leaves as new ones developed. Six ungrafted plants of each variety were grown as controls and two of each variety were kept pruned to two shoots as a check on fruit size. One or more of each graft combination were grown as grafted controls with fruit on both stock and scion shoots and no defoliation (fig. 4).

Table 2 outlines the types of treatment employed in an attempt to obtain effects between stock and scion in a variety of graft combinations. Treatment (a) was employed to study the possible effects on stock and scion fruit in the absence of scion leaves. Scion fruit here would be developed from nutrients transported from leaves of the stock. Treatment (b) provided for the development of scion fruit under the influence of stock leaves and in the absence of stock fruit. Type (c) plants would produce fruit on the scion only, under the influence of both stock and scion leaves. Treatment (d) was used to study the possible effects of stock roots on scion fruits. For this treatment all buds developing below the graft union were removed and plants were trained to one (scion) shoot. Treatment (e) was employed to study possible effects on fruit of both graft symbionts in the absence of stock leaves. Fruit developed on the stock would therefore be under the influence of nutrients furnished by scion leaves. Treatment (f) provided opportunity for studying the development of stock fruit under the influence of scion leaves, but in the absence of scion fruit. Under type (g) stock fruit would be grown under the influence of both stock and scion leaves. Table 1 lists the graft combinations to which the above treatments were applied.

Vitamin analyses.—The fruits, leaves, and roots of the ungrafted control plants and the "d"-treated grafted plants—those consisting only of scion shoot —were analyzed for three of the B complex vitamins: thiamin, riboflavin, and niacin. The "d" plants were selected for analysis as they had no stock shoots which might influence the plant toward the stock variety. Unfortunately, there were not reciprocal "d" grafts for all the five varieties of tomatoes, nor was there more than one plant of some of the "d" grafts. This of course places definite limitations on the conclusions that can be derived from the data since the normal variation in vitamin concentration between individual plants, and environmental influences other than grafting, are not completely eliminated. Nevertheless, certain trends can be indicated by the analysis.

Leaf samples were made by drying twelve to fifteen mature leaves from each grafted plant and

TABLE 2. *Type of treatments employed to obtain reciprocal effects.*

Number of shoots	Fruit production	Leaf treatment	Number plants treated
	Stock on scion effects		
(a) 1 stock, 1 scion	fruit on both	scion defoliated	16
(b) 1 stock, 1 scion	fruit on scion	scion defoliated	30
(c) 1 stock, 1 scion	fruit on scion	no defoliation	14
(d) 0 stock, 1 scion	fruit on scion	no defoliation	14
Total			74
	Scion on stock effects		
(e) 1 stock, 1 scion	fruit on both	stock defoliated	23
(f) 1 stock, 1 scion	fruit on stock	stock defoliated	21
(g) 1 stock, 1 scion	fruit on stock	no defoliation	16
Total			60
	Controls		
Ungrafted			
Shoots not limited	fruit not limited	no defoliation	20
2 shoots	fruit on both	no defoliation	10
Grafted			
1 stock, 1 scion	fruit on both	no defoliation	26
Total			56

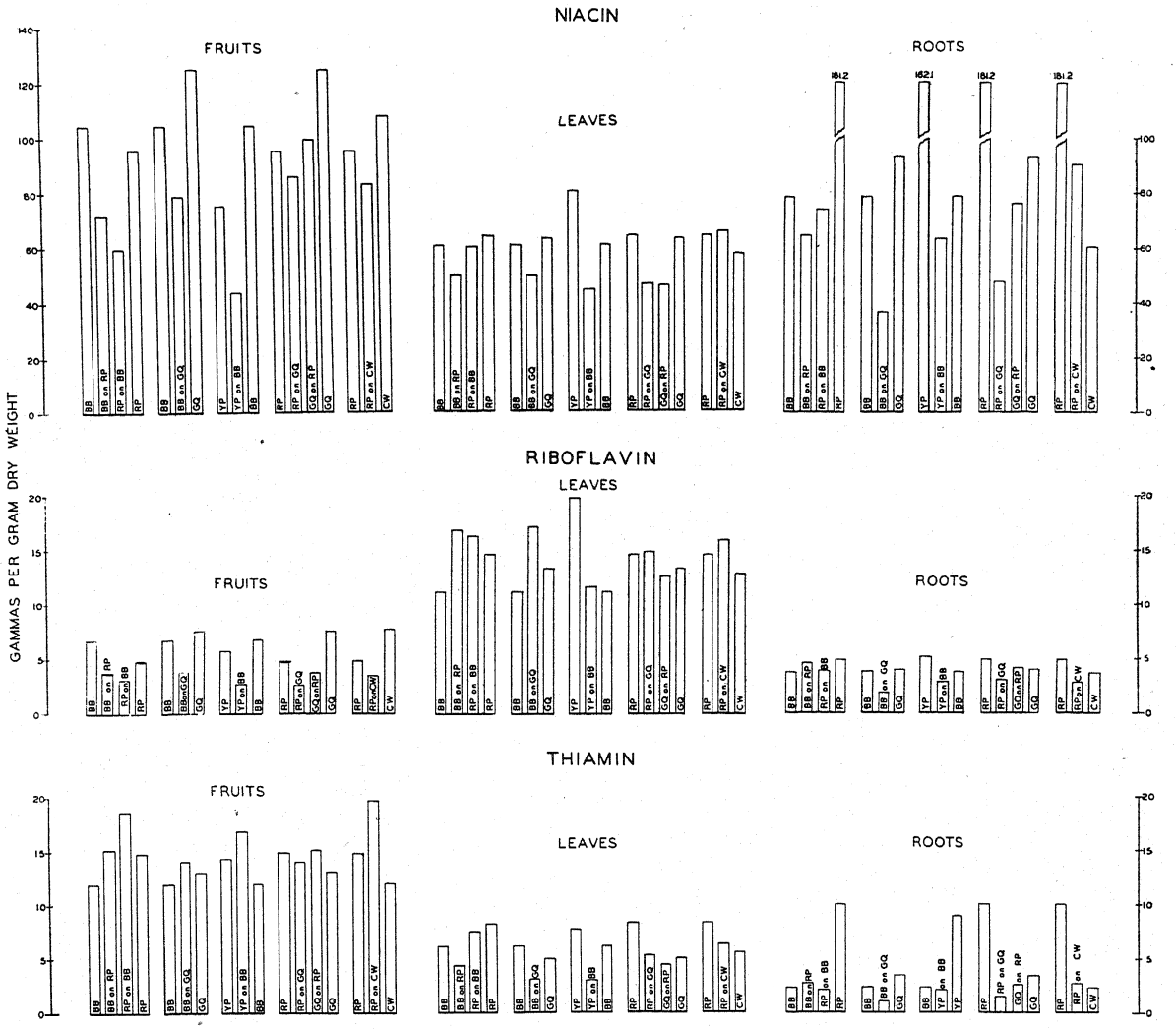


Fig. 5. Bar graph comparing vitamin content in organs of ungrafted tomato plants and types of graft combinations between them. Values given for grafted plants refer to fruits and leaves of the scion and to stock roots. Amounts of niacin, riboflavin and thiamin are expressed in gammas per gram of dry tissue. Bonny Best (BB), Crystal White (CW), Golden Queen (GQ), Yellow Pear (YP), Red Plum (RP).

from six control plants of each variety. Ripe fruits were sectioned and dried so that each sample contained parts of two to six fruits. Roots were dug, washed, and only the younger parts were used for the samples. They were dried for approximately 24 hours at 75°C. in a dehydrator oven with forced circulation. The samples were ground in a Wiley mill and placed in a desiccator in the dark until used.

Extracts of the tissues to be used for vitamin assays were made in a manner similar to that described by Burkholder and McVeigh (1942). A 0.5 g. sample of dry tissue was autoclaved 30 minutes at 15 pounds pressure in 20 cc. of 1 N H_2SO_4. After the extracts cooled and the pH was adjusted to approximately 4.5 with 2.5 N NaOH, they were diluted to 30 cc., filtered, and used. The assays and the preparation of extracts were carried out in red light to prevent the destruction of riboflavin which is photolabile.

The *Phycomyces* assay method (Burkholder and McVeigh, 1940) was used in testing for thiamin. *Lactobacillus casei* ϵ and *L. arabinosus* were used as described by Snell and others (1941) for determining riboflavin and niacin respectively. For measuring the response to riboflavin, the turbidity produced by *L. casei* ϵ, after approximately 24 hours incubation at 37°C., was quantitatively measured in a Klett-Summerson turbidimeter. The niacin results were obtained by titration of the lactic acid produced by *L. arabinosus*, grown for 72 hours at 37°C., with 0.1 N NaOH using brom thymol blue as an indicator. Standard curves for each assay were established with synthetic vitamins in the following concentrations: 0.05, 0.1, 0.15, 0.2, 0.3, and 0.5 micrograms per tube. The amounts of niacin and riboflavin in

the tomato extracts were then read from the standard curves. The results of these assays (table 3 and fig. 5), expressed in micrograms (gammas) per gram of dry tissue are averages of duplicate tubes run at three different levels of the "unknown" extracts.

RESULTS AND DISCUSSION.—*General effects.*—No changes in visible characters were produced in the grafted plants under any combination of treatments employed. The authors were therefore quite unable to confirm the changes in fruit characters of graft symbionts, reported by Avakian and Jastreb, through use of similar techniques on different varieties of tomatoes.

Defoliation and defruiting of either stock or scion produced no change in the number of carpels, size, shape, or color of fruit which could be attributed to grafting. Plants of which one shoot was defoliated produced three to four normal fruits on the defoliated shoot, although the production of new flower buds ceased after leaves had been removed. Development of the young fruit present at the time of defoliation proceeded to normal maturity through the utilization of nutrient materials which presumably passed across the graft union from the other symbiont. Plants consisting solely of stock roots and scion shoots produced normal fruit of the scion type.

Effects on time of fruit ripening in grafts between early and late varieties have been reported (Sahut, 1885; Heinicke, 1927). Of the five varieties employed in this research, fruit of the Crystal White ungrafted controls ripened from 2 to 3 weeks later than that of the other four varieties. Yet in combinations between Crystal White and other varieties there was no evidence of changes in ripening time of either of the graft symbionts.

Although there appeared to be little difference between varieties as to vigor of ungrafted plants, Crystal White proved to be a poor grafting variety. This was particularly true of combinations in which Crystal White was used as the scion, as evidenced by the limited number of successful grafts (table 1).

The grafted control plants developed normally with no modification of fruit characters. Each graft symbiont produced fruit characteristic of its own variety, with the result that every plant consisting of two shoots produced two kinds of fruit. This was also true of graft combinations with maximum differences in fruit characters, as in reciprocal grafts between Red Plum and Golden Queen (fig. 4).

Vitamins.—Results of the vitamin analyses for the five varieties which were assayed are given in table 3. The vitamin results are expressed as micrograms of vitamin per gram of dried tissue so that all the data are comparable. It is evident that roots, fruits and leaves of tomato plants differ from each other as to content of these three vitamins, and that concentrations of thiamin, riboflavin and niacin differ within each organ. Thiamin and niacin are apparently made or stored in large quantities in the fruits and may therefore be related to seed development. The concentration of riboflavin in the leaves is twice as great as elsewhere, which may mean it is synthesized or accumulated there. Niacin, however, is much more abundant in roots than in leaves, indicating possible synthesis or storage of niacin by roots.

A comparison between organs of different varieties also indicates the existence of varietal variation in vitamin content. With the exception of riboflavin and niacin in fruits, vitamin concentration is higher in Red Plum and Yellow Pear (the two small fruited varieties) than in Golden Queen, Bonny Best, or Crystal White. It is therefore important to determine whether these varietal differences are reflected in the vitamin content of grafted plants.

The grafting generally causes a reduction in vitamin concentration, and has little or no effect toward raising the vitamin level of either stock or scion tissues (fig. 5) when a "low-vitamin" variety is grafted with Red Plum or Yellow Pear. The grafted plants, though apparently as vigorous as the controls, have lower concentrations of vitamins, with the exception of thiamin in fruits and possibly ribo-

TABLE 3. *Thiamin, riboflavin, and niacin content (expressed in gammas per gram of dry tissue) in fruits, leaves, and roots of Bonny Best (BB), Crystal White (CW), Golden Queen (GQ), Yellow Pear (YP), and Red Plum (RP) varieties of tomatoes.*

	BB	CW	GQ	YP	RP
Niacin					
Roots	79.0	61.6	93.1	162.1	181.2
Leaves	60.8	57.6	62.8	82.7	64.0
Fruits	104.7	108.4	125.0	75.3	95.8
Thiamin					
Roots	2.4	2.0	3.5	9.2	10.3
Leaves	6.0	5.5	4.9	7.7	8.0
Fruits	12.0	12.1	13.2	14.3	14.8
Riboflavin					
Roots	3.8	4.2	4.0	5.3	5.0
Leaves	11.4	13.1	13.7	19.0	15.0
Fruits	6.8	7.8	7.7	5.8	5.0

flavin in leaves, than do the control plants. Certain compounds elaborated in one part of the plant and transported to another, for manufacture of vitamins in that part, may have been hindered by grafting together two varieties of tomatoes which were not alike in their ability to synthesize vitamins or their precursors. This seems likely to be the case for niacin, which drops off consistently in the grafted combinations (fig. 5). When, for example, Bonny Best variety is grafted to Red Plum, the niacin content of Red Plum roots is lowered and approaches the level in roots of ungrafted Bonny Best plants. The same is true of thiamin. Roots are perhaps dependent upon precursors supplied by the leaves. However, a reciprocal effect could not be demonstrated. When, for example, Red Plum variety is grafted to Bonny Best, the niacin concentration in Bonny Best roots, instead of being raised by the Red Plum leaves, remains low (fig. 5). Perhaps synthesis of vitamins cannot be increased above the normal maximum of the graft symbiont with the lowest vitamin concentration. On the other hand, the synthesis of vitamins by the graft partner which normally produces the most vitamins may be limited by the precursors which the other partner can produce. These same problems are apparent regarding the other vitamins and other graft combinations.

SUMMARY

Five varieties of tomatoes were reciprocally grafted, and the effects of defoliation and defruiting of the graft symbionts were observed. No changes in visible characters of the grafted plants were produced which would indicate a reciprocal effect between stock and scion under any combination of treatments employed.

Analyses for thiamin, riboflavin, and niacin were made on fruits, roots, and leaves of ungrafted and grafted plants. Varietal differences in vitamin content and distribution were demonstrated. Fruits contained the greatest amount of thiamin and niacin. Leaves were highest in riboflavin, and roots contained more niacin than leaves. In general, vitamin concentrations were lower in organs of grafted than in those of ungrafted tomato plants, and no evidence was obtained that differences between two graft symbionts in vitamin production were transferred from one moiety to the other.

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GENETICS OF GLOMERELLA. IV. NUCLEAR PHENOMENA IN THE ASCUS [1]

George Blanchard Lucas

IT HAS BEEN shown that in certain fungi belonging in the genus *Glomerella* (Lucas, Chilton, Edgerton, 1944; Edgerton, Chilton, Lucas, 1945; Chilton, Lucas, Edgerton, 1945) there is an interesting association of strains. These strains differ morphologically and physiologically. When certain of these strains grow together on nutrient media in a petri dish, a cross fertilization occurs on the line of contact and perithecia form in great numbers. Ascospores have been isolated in large numbers from the perithecia on the line of contact between strains and also from perithecia produced by individual strains.

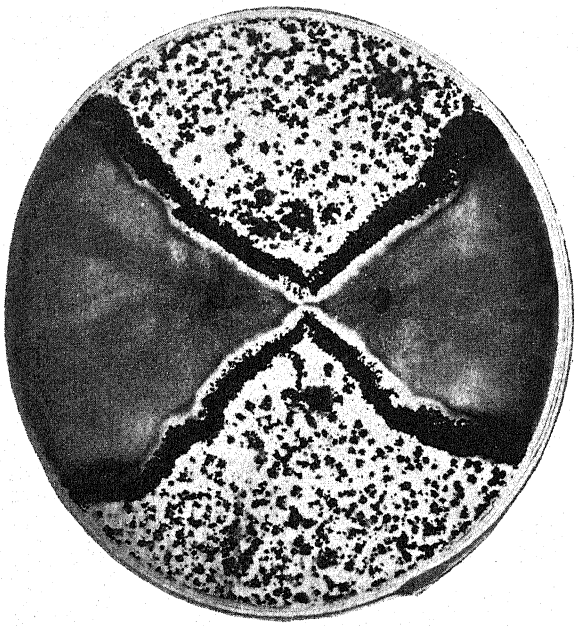


Fig. 1. Ridge of perithecia on line of contact between plus B and conidial B strains. Above and below, plus B; right and left, conidial B.

In studying these isolates, certain results have been obtained which as yet have not been explained satisfactorily. When ascospores are isolated from one of these strains (plus A) which is presumed to be haploid, cultures of two types (plus A and minus A) are practically always obtained. Also when ascospores are cultured from the line of contact between strains, two or more strains are often obtained from ascospores of a single ascus but sometimes all eight ascospores produce cultures of a single strain. No satisfactory explanation of this distribution of strains developing from ascospores of individual asci has been presented as yet.

Seeking the solution of some of the problems by a different approach, a study of the cytology of *Glomerella* is now being made. Such a study should also be of aid to those mycologists who are interested in the cytology, development, and relationships of the fungi.

Glomerella is an excellent fungus for a cytological study. The perithecia begin to form in a few days after transfers are made to agar plates. The perithecia develop in great numbers and are very close together. Perithecia of all ages occur together, so it is possible to have perithecia of various ages on a single slide. Also asci in various stages of development occur in the same perithecium.

The present paper is confined to the nuclear fusion in the ascus and the subsequent three divisions preceding the cutting-out of the ascospores.

LITERATURE REFERENCES.—That there are not more papers dealing with nuclear phenomena in the ascus is doubtless due to the difficulties involved in working with the Ascomycetes and more especially the Pyrenomycetes. The chromosomes are extremely small, the divisions in the ascus follow each other rapidly and the obtaining of abundant material in the right stages of development is often difficult.

There are, however, a number of papers, mostly published in recent years, in which the relations between the genetics of certain fungi and the nuclear phenomena are discussed. Only those which seem to have a special bearing on the present paper are being listed.

In an early paper, Harper (1905) presented studies on some of the powdery mildews. He reported a conspicuous central body in the nucleus to which the chromatin material was attached. In later years, Lindegren (1945) reported on *Saccharomyces,* Colson (1934) and McClintock (1945) on *Neurospora* and Backus and Keitt (1940) on *Venturia inaequalis*. Points of interest in these reports include the presence or absence of a centrosome, and the changes in the nucleolus and the chromosomes in the various divisions in the ascus.

MATERIALS AND METHODS.—The perithecia of *Glomerella* used for sectioning and staining were produced by pure cultures on agar media in petri dishes. Blocks of agar containing the perithecia were fixed in weak Fleming's solution and later imbedded in the usual way. Cultures 5 to 14 days old were used. Most of the sections were cut 6 μ thick. While various stains were used, the crystal violet procedure as recommended by Sass (1940) was found the most satisfactory. The sections were stained with a one per cent aqueous solution of crystal violet and destained with picric acid and clove oil.

Three different strains of *Glomerella* were used: 5–16–1 (plus B), 17–F–5 (minus B), and 36–D–3 (conidial B). These three strains have been previously described (Edgerton, Chilton, and Lucas, 1945; Chilton, Lucas, and Edgerton, 1945). The plus B strain forms perithecia in scattered, glom-

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erate masses, the minus B strain produces numerous perithecia but scattered singly or in groups of two or three over the surface of the agar and the conidial B strain produces conidia in abundance but no perithecia. All of these strains had previously been isolated from the progeny of a culture of the plus type obtained from a dead stem of *Ipomoea*. This fungus is being considered as belonging to the complex and variable species, *Glomerella cingulata* (Stoneman) Spauld. & v. Schr.

Perithecia used for sectioning included ones produced by the plus B strain and by the minus B strain, and also ones produced on the line of contact between colonies of the plus B and the conidial B strains. Previous unpublished investigations had indicated that the perithecia on the line of contact between the plus B and the conidial B strain (fig. 1) developed following fertilization between the strains.

For illustration, it has seemed better to depend on photomicrographs rather than drawings. The chromosomes are extremely small and even with the highest powers of the microscope, it was not easy to be sure of details. All photomicrographs were made at 1925 magnification, using a 100 X, Fluorite oil immersion objective and a 10 X eyepiece, and with a bellows length of 20 inches.

Since the details of the nuclear divisions in all perithecia were observed to be strikingly similar, the following descriptions apply equally well to asci of the plus and minus strains and to the crosses in which the conidial B strain was used.

PERITHECIUM AND ASCUS FORMATION.—While the perithecium is young and developing, the internal area is filled with closely-packed delicate hyphae. Eventually, however, from the fertile region at the base of the perithecium, the asci arise and finally fill this central area. The branching of the hyphae in the fertile region appeared complicated and no attempt was made to follow any one hypha. It cannot be stated whether or not all the hyphae in this fertile region were ascogenous hyphae. No crozier formation was distinguished but it is not clear whether these structures can be brought out with the crystal violet stain, which does not seem to stain well structures other than chromosomes and nucleoli. No paraphyses were seen pushing up from the base of the perithecium.

FUSION OF NUCLEI IN ASCUS.—The young ascus (ascus to right in fig. 2A) was observed to contain two nuclei which were assumed to be haploid nuclei. The most conspicuous feature of these nuclei was the large, spherical, deeply-staining nucleolus. This nucleolus was surrounded by a clear, hyaline area. That these two nuclei fused seemed clear from the studies that were made.

While it was not possible to follow all details of the fusion, it appeared that as the two nuclei prepared to fuse, the chromatin material became visible and the nucleolus of each increased in size. Soon the spiremes became evident and one large chromosome was observed attached to the nucleolus. At this stage, the two nuclei came to lie side by side, with the nuclear membranes in contact. Later, the nuclei presumably fused and the chromosomes from the two nuclei intermingled. Eventually the two nucleoli joined to form a large "fusion nucleolus" which was approximately twice the diameter of the haploid nucleolus. That this was a fusion nucleolus was further indicated by the fact that frequently two chromosomes were observed attached to it, presumably one coming from each nucleolus (fig. 2B). Measurements of thirty of these fusion nucleoli showed them to range from 1.4 to 1.6 μ in diameter. The fusion nucleoli were remarkably consistent in size.

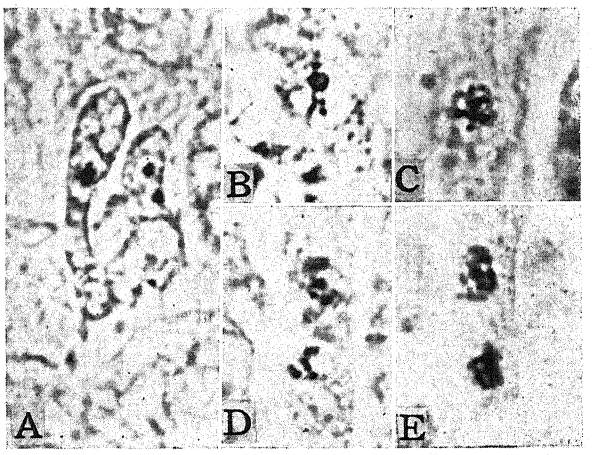


Fig. 2. A. Young asci showing nuclei before fusion. Note conspicuous nucleoli. B. Fusion nucleolus with two attached chromosomes. C. Prophase I (leptotene). Nucleolus has disappeared. D. Telophase I. E. Prophase II.

The fusion nucleus was quite large and conspicuous. Its diameter was just slightly less than that of the ascus. Frequently the chromatin material seemed scattered around the periphery of the nuclear area, but more often the spiremes were clearly evident, twisting and coiling throughout the nuclear area. Sometimes, too, the spiremes appeared to be clustered or aggregated at one side of the nucleus with the nucleolus at the other side and connected to the aggregate by a single spireme. At this stage (leptotene), the chromosomes reached their maximum length. However, a centrosome, "central body" or centriole to which the chromosomes were attached was not observed, or at least not recognized in any of the fusion nuclei.

Studies of the nuclei at this stage gave some indication of the relative lengths of the chromosomes. Of the chromosomes which could be distinguished, the one to which the nucleolus was attached, the nucleolus chromosome, appeared to be the longest and was approximately 4 μ long at prophase I. It is designated as chromosome 1. Chromosome 2 was slightly shorter. Chromosome 3 was approximately one-half the length of chromosome 1, while chromosome 4, the shortest of all, was perhaps no more

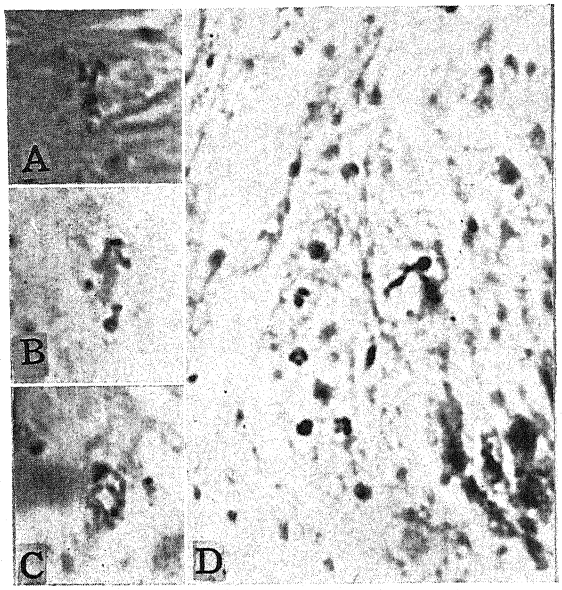


Fig. 3. A. Metaphase I. B. Prophase I. C. Prophase I, nucleolus still evident. D. Ascus to left, third division (telophase); ascus to right with fusion nucleus. Note difference in length of chromosomes in two asci.

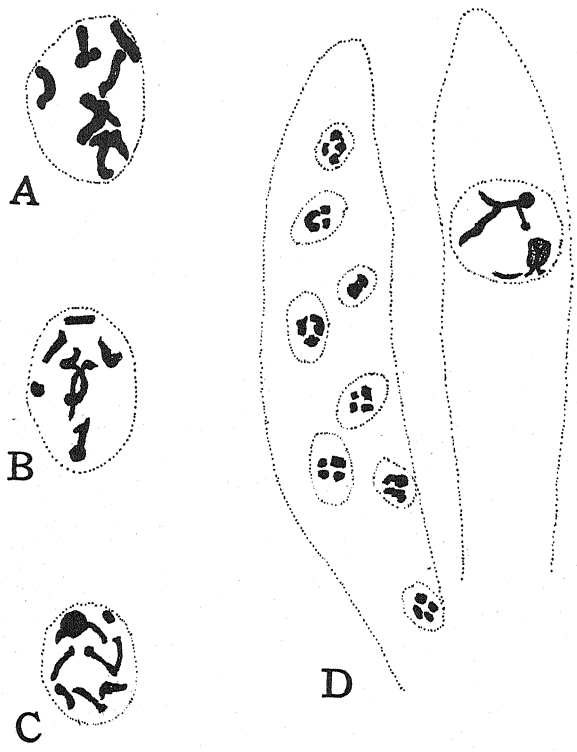


Fig. 4. Diagrammatic sketches of nuclei shown in fig. 3.

than 1 μ in length. At this stage, also, the ascus was apparently at its full length.

Gradually, the fusion nucleolus disappeared and only the spiremes were evident (fig. 2C). The chromosomes became associated in pairs at synaptotene and began to shorten and thicken at the pachytene stage. At this stage, there appeared to be eight chromosomes, although it was difficult to count them with certainty (fig. 3A, B, C, and 4A, B, C). At least, no figure was seen with more than eight chromosomes.

First division in ascus.—The first nuclear division in the ascus occurred along the longitudinal axis of the ascus (fig. 2D). No figures of division I were observed in which spindle fibers were clearly evident, although figures were observed which resembled some in *Neurospora* as presented by Colson. Frequently the chromosomes at late anaphase or early telophase appeared to be located in a clear vacuolar area. No nucleoli were visible after metaphase I and it seems probable that the chromosomes passed from telophase of division I to prophase of division II without the nuclei being completely reformed. At least, no such figures were noticed in the rather large number of asci observed. This indicated, too, that division II closely followed division I. At early telophase of division I, figures were observed which indicated the haploid number of chromosomes to be four (fig. 2D).

Second division in ascus.—The second nuclear division in the ascus occurred either longitudinally or diagonally. Figures of division II occurred much less frequently than the fusion nucleus stage. At telophase I, the chromosomes were observed to be shortened and thickened, but at prophase II, they had again elongated and were about the same length as in prophase I (fig. 2E). However, they appeared thinner and more bead-like than in prophase I. At anaphase II, the four groups of chromosomes were sometimes located at some distance from each other (fig. 5A), and sometimes, they occupied the center of the ascus grouped closely together (fig. 5B). In some asci they appeared to be located in a clear area while in others they were not. No prominent spindles were seen but figures suggestive of spindles were observed. In all cases, these structures were located in vacuolar areas. No nucleoli were seen in figures of division II and no figures were seen in which the daughter nuclei reformed with chromatin strands and nucleoli as has been reported in several other fungi. It seems probable that division II was followed very quickly by the third division and possibly the chromosomes passed directly from telophase II to prophase of the third division. Because the chromosomes were so small and thin, it was not possible to detect any apparent doubleness of the chromosomes.

Third division in ascus.—As stated above, the third division in the ascus quickly followed division II. Due to the absence of conspicuous spindles, it is difficult to state with certainty whether the divisions were transverse or diagonal. However, by comparing the appearance of the chromosome groups, it sometimes seemed possible to determine sister groups of chromosomes. This was possible because in some asci, the chromosomes at late anaphase or telophase of the third division were

grouped in such a way that chromosome 4 was visible as a small round dot. By noting the position of chromosome 4 in adjacent chromosome groups, it was possible to determine which groups had been derived from the same nucleus of division II.

The chromosome groups at telophase of the third division were seen to form in several different patterns. Most frequently, they appeared as two evenly biseriate rows with the pairs of nuclei directly opposite each other, four in each row, or diagonal to each other in two rows of four each.

In the third division, the chromosomes frequently appeared to be located in a hyaline or vacuolar area. In many instances, four distinct small bodies which were assumed to be the chromosomes distributed in the form of a tetrad were clearly visible (fig. 5C). At this stage, these chromosomes were extremely small, being no more than 0.6 μ in length. The chromosomes were clustered so close together at late telophase that if a preparation was slightly overstained, the chromosome groups appeared as round, black masses (fig. 5D). In such figures, each group of overstained chromosomes had the appearance of, and was about the same diameter as a fusion nucleolus.

ASCOSPORE FORMATION.—At late telophase of the third division, the nucleolus of each ascospore nucleus began to appear. As the chromosomes began to lose their conspicuous staining, the nucleolus became more prominent. Generally coincident with the appearance of the nucleolus was the delimitation of the ascospore. At this stage, the chromosomes were very small. Eventually the chromosomes disappeared and in the mature ascospore (fig. 5D) only the nucleolus itself stained. At this stage, the nucleolus was approximately 0.5 μ or less in diameter. How the ascospore walls were delimited was not determined.

DISCUSSION.—From the studies which have been made, several things seem important and perhaps should be emphasized.

It is significant that the chromosome number and the details of the nuclear divisions in the ascus were so similar that it was not possible from the stained sections to say whether the perithecia came from the plus B strain, the minus B strain or from the cross between the conidial B and the plus B strains.

In the many asci observed at prophase I and metaphase I, no figure was seen which contained more than eight chromosomes and numerous asci were seen in the third division in which four chromosomes were clearly and distinctly visible. This indicates that in the ascus of *Glomerella,* there is but a single reduction of chromosomes; that is, the first two divisions in the ascus are meiotic and that the third division is equational and not brachymeiotic. This also indicates that the fusion nucleus in the ascus is diploid (not tetraploid), that the ascospores are haploid and that only one nuclear fusion occurs. This fusion evidently takes place in the young ascus.

The nucleolus in the ascus of *Glomerella* undergoes certain changes which seem to be slightly different from that which has been reported for certain other Ascomycetes. Based on the stains and the technique used, the evidence indicates that the nucleolus disappears at late prophase of division I and does not reappear, or at least it does not become recognizable again, until it is reformed at the telophase of the third division when the ascospores are being formed. As reported in the literature, in some Ascomycetes the nucleolus seems to persist through at least the first division in the ascus and sometimes the second; in some cases, as in *Neurospora,* the nucleolus becomes detached and can be seen in the cytoplasm; while in other cases the nucleolus is reconstituted again in the daughter nuclei at telophase I and at telophase II.

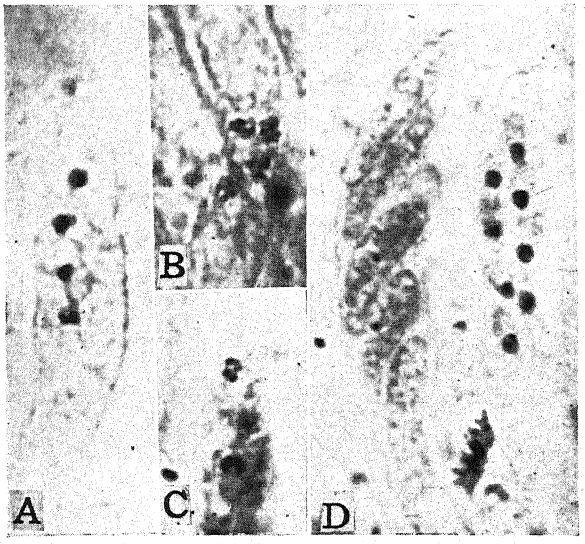


Fig. 5. A. Telophase II, division along longitudinal axis of ascus. B. Telophase II, division along transverse axis. C. Telophase of third division with four chromosomes evident. D. Ascus to left with mature ascospores, showing the small nucleoli; ascus to right, telophase of third division.

The chromosomes also exhibit a remarkable increase and subsequent decrease in length and thickness during the various divisions. At prophase I the chromosomes are quite long and conspicuous, the longest being at least 4 μ. By metaphase I, they are shorter and thicker. At prophase II, they are again elongated but are perhaps not quite as long as in prophase I. At anaphase II, however, they are markedly decreased in both length and width and at the telophase-stage of the third division, they have shrunken to almost the limits of visibility, the longest being no more than 0.6 μ in length. These changes in length must take place very rapidly.

SUMMARY

Perithecia of *Glomerella* formed by mating certain strains and perithecia formed by some of these

strains growing alone were sectioned and stained with crystal violet.

Asci were seen in all stages of development. No difference in appearance of the asci could be observed, no matter whether they arose as a result of a cross or from individual strains growing alone. Each young ascus as it formed contained two haploid nuclei. These fused to form a large fusion nucleus which contained a large and conspicuous fusion nucleolus. This fusion nucleolus seemed to disappear before metaphase I and was not evident until after the third ascus division occurred, at which time a small nucleolus appeared in the nucleus of each mature ascospore. Two chromosomes were seen attached to the fusion nucleolus, which was interpreted to mean that fusion had taken place. It is assumed that each of the two chromosomes was associated with its own nucleolus previous to nucleolar fusion.

The three divisions in the ascus followed each other rapidly. At prophase I, the chromosomes reached their maximum length. At this stage, the chromosomes to which the nucleoli were attached were about 4 μ long. At telophase of the third division the chromosomes were much reduced in size, the longest being no more than 0.6 μ in length. The indications are that the haploid number of chromosomes is four and the diploid eight. Apparently only one fusion and one reduction of the chromosomes occur.

The divisions in the ascus of *Glomerella* seem to be anastral. No central body to which the chromosomes might be attached was observed. No centriole or astral rays were seen. Spindle formation was not observed. The divisions appeared to be intranuclear. Division I occurred in the longitudinal axis of the ascus. Division II was either longitudinal or oblique while the third division was either transverse or oblique. Mature ascospores of *Glomerella* were uninucleate.

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ONTOGENY OF THE SCLEREIDS IN THE INTEGUMENT OF PISUM SATIVUM L.[1]

R. M. Reeve

During maturation of integumentary tissues in peas of commercial harvesting ages, the secondary walls of the macro- and osteosclereids (epidermis and hypodermis, respectively) become specialized with fluted and twisted thickenings. These are very pronounced in the mature macrosclereids. Composition, optical properties, and the orientation of cellulosic fibrils of these secondary walls have been investigated, and the results of tests and observations on maturity changes have been reported in a previous paper (Reeve, 1946a). Histochemical tests show that these specialized thickenings are composed of a pentosan-cellulosic complex which is of relatively high resistance to extraction methods. The significance of this chemical composition and the relationships of microfibrillar structure and crystallite orientation to texture previously have been discussed (Reeve, 1946a, 1946b).

In general, the degree of crystallite orientation, the pentosan content, and the amount of wall thickening increase with maturation. These increases are most pronounced within the range of ovule sizes found in peas of commercial harvest ages suitable for food processing. The combination of these factors influences the degree of toughness or the inherent texture quality of the tissues. In addition, new technological developments in food processing are apt to affect texture and other factors of quality in processed foods. Histological information on the inherent factors of texture is necessary to an understanding of how and to what degree the tissues are affected. Such information also is fundamental to the genetical development of new varieties having characteristics necessary to the main-

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tenance and improvement of quality in the processed food product.

The present paper describes the development of the sclereids in the integuments of peas mainly prior to harvest age, and relates histogenesis to the general pattern of ovule growth. Several garden varieties were utilized in these investigations, but, because no significant differences between varieties occur in the ontogeny of the sclereids during the early stages of ovule development, there is no attempt here to make comparisons. For convenience, height in millimeters is used for reference to ovule size in the early stages, and commercial sieve size is used for the later stages in which the ovule tends to develop a spherical shape. Sieve size one is $^9/_{32}$ inches diameter, and each additional size is an increase of $^1/_{32}$ inches diameter. The use of these size indices is merely to provide an approximate relationship of size to histological development. "Optimum" sizes in commercial harvest range from two through five, but there is considerable variation in quality and maturation in both large and small seeded varieties because of factors of environment. In general, within the commercial harvest sizes, the tissues of the small seeded varieties apparently mature more rapidly and perhaps more uniformly with reference to size than do some of the large seeded varieties. However, this subject requires detailed investigation before any definite histological characteristics can be safely applied to varietal comparison.

HISTOGENESIS OF THE INTEGUMENT.—*Formation and early differentiation.*—The initiation and early formation of the angiosperm ovule as a nucellar primordium, the development of its integumentary folds, and its embryogeny have been widely investigated. These events are well summarized for the ovule of *Pisum sativum* by Hayward (1938). There have been few investigations on tissue differentiation in the ovule wall of legume seeds, the most detailed being Zimmerman's (1936) studies on the development of the strophiole. There have been no complete studies on the ontogeny of the sclereids.

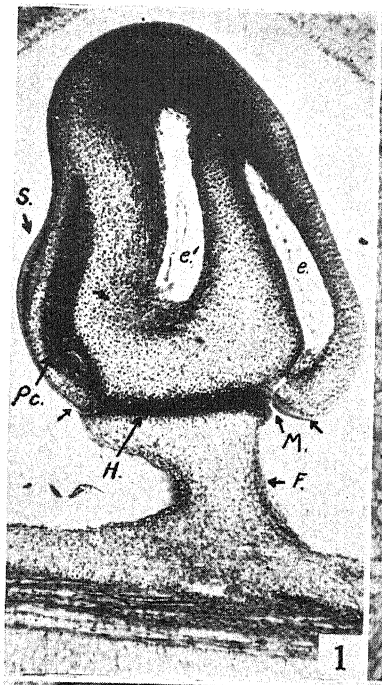
The first stage of tissue differentiation treated here may be best described as that represented by an ovule in a young pod shortly following pollination and about the time the outer flower parts have withered and the ovary has protruded beyond the collar of diadelphous stamens. Fig. 1 to 3 show three successively older ovules in three different planes of section (see legend), but representing approximately this first stage of zonal differentiation of the tissues. In fig. 1, the ovule wall is not distinct from the integuments except toward the micropyle. The epidermal layer is well defined, but is in approximately the same meristematic condition as found in the unfertilized ovule, and the hypodermis is yet undifferentiated (compare with fig. 8). Fig. 2 and 3 show slightly more advanced stages in which the zonal differentiation is slightly more pronounced. For convenience the area of the testa not associated with such specialized structures as the strophiole and micropyle, are referred to as the "lateral sides" as shown in fig. 2 and 3.

In the stages of development just described there is no evidence of periclinal divisions in the epidermal layer of the integument. In earlier stages, as represented by unexpanded flowers, virtually the same condition exists, *i.e.*, divisions in the epidermal layer are only anticlinal. With the possible exception of periclinal divisions in the protoderm of the nucellar apex, forming an "epidermal cap" at the time of archesporial differentiation (Hayward, 1938), it seems apparent that the protoderm of the integument remains discrete throughout its ontogeny. The hypodermal layer, on the other hand, does not become differentiated to form osteosclereid initials until the protoderm cells have begun to elongate. This occurs at about the stage represented by fig. 3 (compare with fig. 11–16).

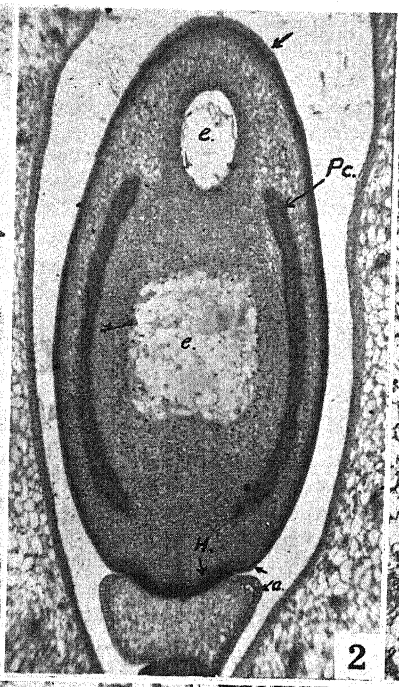
Regional differentiation.—Upon examination of longitudinal, median sections of very young ovules it is clearly evident that the epidermal layer of the integument undergoes a more rapid differentiation in some regions than in others. The earliest stages of this regional specialization probably begins before fertilization. When the ovules are between 1 and 3 mm. high, the elongation of the macrosclereids is very pronounced in the strophiolar area where their total lengths may be ten to fifteen times as great as the lengths of adjacent epidermal cells (compare fig. 1–3, and 10–16). In these stages the epidermal cells overlying the "top" of the ovule also are somewhat elongated, and the young macrosclereids of the micropylar region are further differentiated than those in the lateral sides of the ovule.

At these early stages of ovule development, the hilum region is still highly meristematic in contrast to the differentiating parenchyma cells of the rest of the ovule wall (fig. 1 and 4–6). In the maturing seed the hilum region becomes specialized with two layers of macrosclereids and an underlying pocket or "tracheid island" of cells with lignified, reticulated wall thickenings (fig. 6 and 7). Some authors have referred to the "tracheid island" as "reticulate sclerenchyma," and the latter term was used in a previous paper (Reeve, 1946a). This tissue was described by Tschirch and Oesterle (1893–1897) as a "tracheid island." The term "sclerenchyma" ordinarily is not applicable to cells either of "tracheary" structure or origin (Foster, 1942).

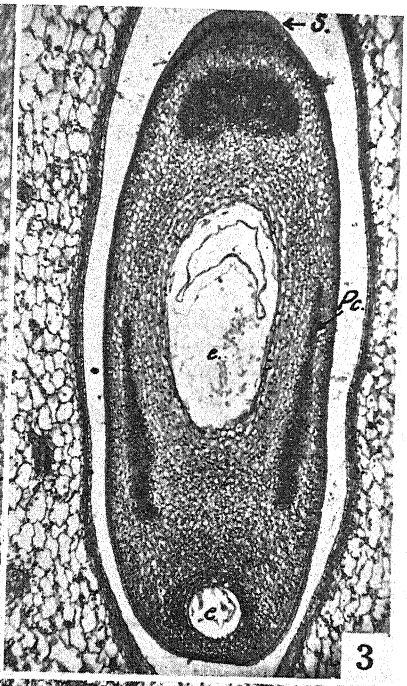
At the end of the hilum distal to the micropyle the procambium of the ovule differentiates away from the vascular tissues of the placenta, through the funiculus, and into the young ovule in the region which underlies the strophiole. Careful examination of both longitudinal and transverse serial sections shows that elongation of the macrosclereids in the hilum region progresses from all sides towards the center of the area. However, this differentiation appears to proceed more rapidly from the strophiolar end than from elsewhere (fig. 4–6).



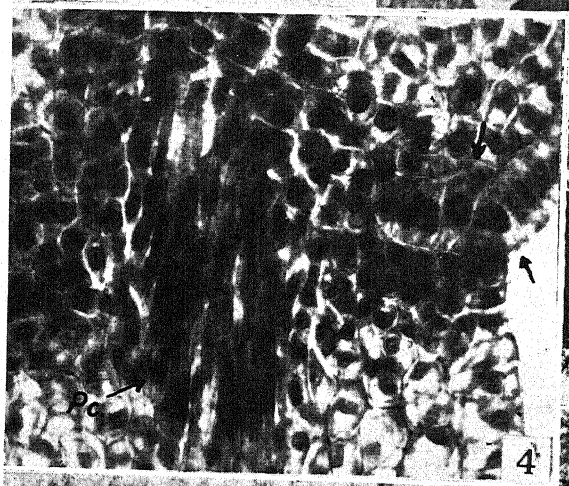
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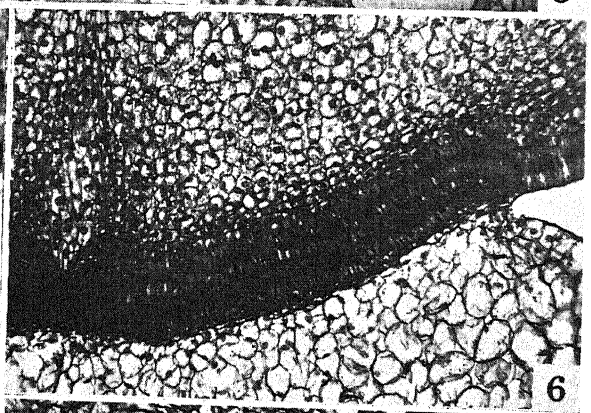
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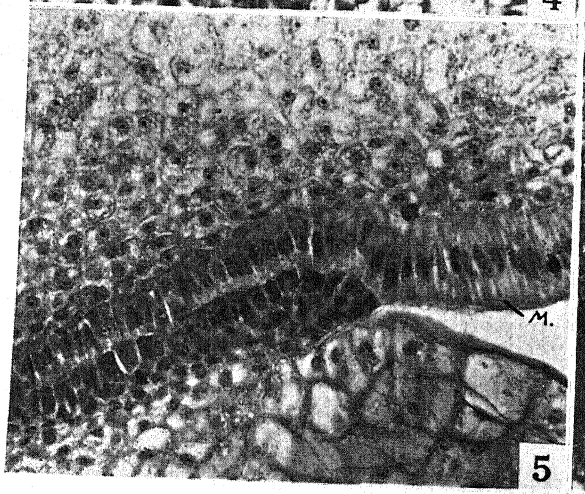
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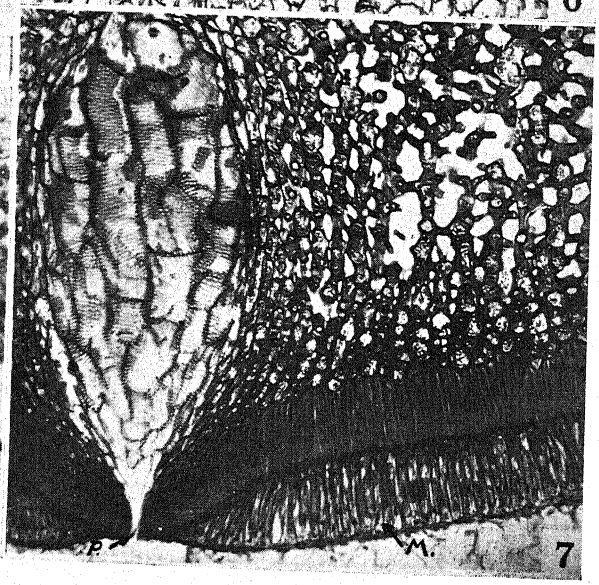
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To all appearances, the tissue zones of the hilum region are the last to become well differentiated, and although the area is a small part of the total surface of the seed, the degree of its specialization justifies further consideration here.

Differentiation of the hilum region.—Pammel (1899) considers both rows of the macrosclereids which develop over the hilum to be a part of the testa. In the light of information presented here, his interpretation is logical and correct. Obviously, however, the entire hilum area in its meristematic condition contains no definable protoderm as is already differentiated over the rest of the testa. One can only speculate as to the physiological and inherent causes for the formation of the double layer of macrosclereids over the hilum from ground meristem in contrast to the formation of macrosclereids from the protoderm over the rest of the testa.

In some leguminous seeds there is an arilloid process adjacent to the hilum, but in most leguminosae the arillus is an appendage of the funiculus. In *Pisum* it is thought to function as a pinching-off mechanism which aids in the detachment of the ripened seed (Pammel, 1899), and occurs as an outgrowth of the funiculus (fig. 2).

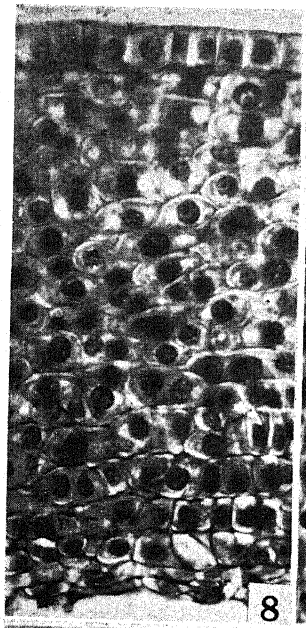
When the young ovule is about 2mm. high the hilum tissues are still meristematic and show little differentiation except at the margin, where macrosclereids are differentiating, and in the development of the procambium of the ovule bundle (fig. 1). At this stage, the immature cells of the parenchyma above and below the hilum have undergone some enlargement and vacuolation. By the time the ovule is slightly larger, these parenchymatous cells have enlarged considerable, have become vacuolate, and contain numerous small starch grains (fig. 4, 5 and 6). Within this period of growth the double row of macrosclereids has differentiated almost to the center of the hilum, and it can be seen that one could regard the outermost layer as originating from the funiculus (fig. 5 and 6). In certain histogenetic features, the hilum area is analogous to the abscission tissues of the leaf petiole, but it is significant that both rows of macrosclereids remain with the testa of the detached, ripened seed.

Early differentiation of the macrosclereids over the hilum is virtually complete before there is any pronounced differentiation of the "tracheid island" (fig. 6), the cells of which are the last to become well-defined as a tissue zone. In an ovule about 3 mm. high, this "island" appears as a more or less meristematic tissue composed of vacuolating cells which contain less starch than the surrounding parenchyma. By the time the ovule has reached harvestable age (sieve size one or two), the reticulate thickenings of the walls of these cells are evident. At this stage many of the cells of the "island," particularly at the margin of its area away from the macrosclereids, are still in early stages of differentiation and show no evidence of reticulate wall structure. The earliest formed cells of the "tracheid island" are those in close proximity to the center of the hilum area. Here, in older ovules, the "tracheid island" narrows into a keel which protrudes slightly into the minute pore or slit where the macrosclereids have not completely sealed off the hilum; in transverse sections of the hilum this keel appears funnel shaped (fig. 7). Sections cut parallel to the long axis of the hilum show some connection, through transitional forms of tracheary elements of the "tracheid island," with the xylem of the ovule bundle. Pammel (1899) has discussed these tissues in the mature seeds, and other workers have utilized their specializations in structure to explain various physiological processes or mechanisms during dormancy and in seed germination.

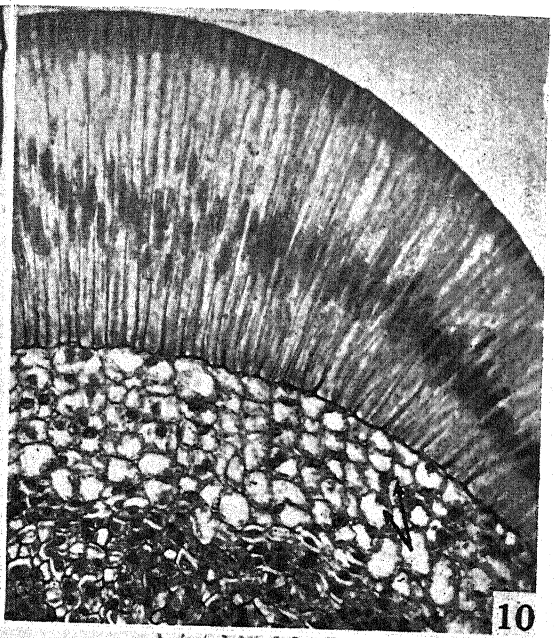
In some genera it is apparent that the micropyle and the hilar groove (Pammel, 1899) become closed by growth of the macrosclereids. Thus most of the seed coat in these is fairly impervious to water. Hamly (1932) investigated the seed of *Melilotus alba* with reference to softening for germination. In this species the strophiolar region develops a cleft or groove, apparently of schizogenous origin, through the extremely elongated macrosclereids. Hamly was able to demonstrate that the softer seeds were naturally permeable through this cleft, and that in harder seeds the cleft could be mechanically produced by impact or opened by moderate heating. In *Pisum* there is no strophiolar cleft, but it would appear that the hilar groove which opens into the "tracheid island" provides sufficient passage for gases and water.

Immediately surrounding the "tracheid island" in the mature seed is a layer of thin-walled, somewhat elongated parenchyma cells. Sometimes these

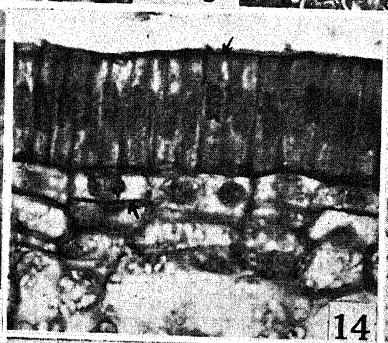
Fig. 1–7.—Fig. 1–3. Young ovules section in three different planes showing early regional differentiation of testa. "a" = arillus, "e" and "e'" = embryosac, "F" = funiculus, "H" = hilum, "M" = micropyle, "Pc" = procambium, "S" = strophiole.—Fig. 1. Median longi-section parallel long axis of hilum; note early elongation of macrosclereids of strophiole. Ovule about 1½ mm. high. ×40.—Fig. 2. Longi-section perpendicular to long axis of hilum; note greater elongation of macrosclereids at top of ovule. Ovule slightly over 2 mm. high. ×40.—Fig. 3. Trans-section of ovule 2½ to 3 mm. high, cut through strophiole and above micropyle; note greatly elongated macrosclereids of strophiole. ×40.—Fig. 4–7. Progressive stages of differentiation of hilum region.—Fig. 4. Cut in plane at right angles to long axis of hilum; ovule about same age as in fig. 1. ×480.—Fig. 5. From ovule slightly older than that shown in fig. 1; and section perpendicular to long axis of hilum; note vacuolation and starch of cells above and below hilum; innermost layer of macrosclereids differentiates slightly in advance of outermost layer. ×240.—Fig. 6. Section from ovule same age as in fig. 2, and cut perpendicular to long axis of hilum, with macrosclereids still meristematic; note early differentiation of "tracheid island" on the left. ×120.—Fig. 7. Section from ovule representative of stage found in sieve size one, and cut in plane perpendicular to long axis of hilum; note particularly the tissue surrounding the "tracheid island"; "p" = pore or hilar groove, "M" = outer layer of macrosclereids of hilum. ×120.

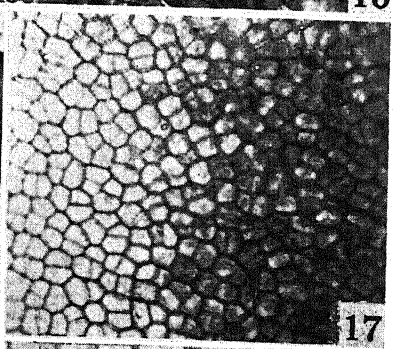

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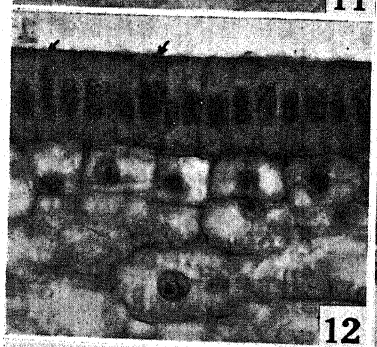

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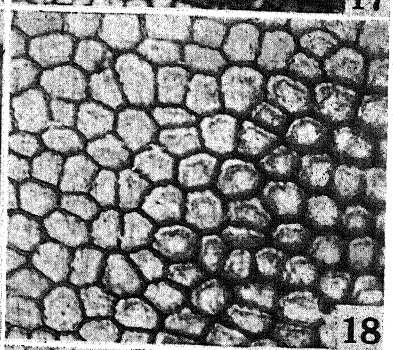

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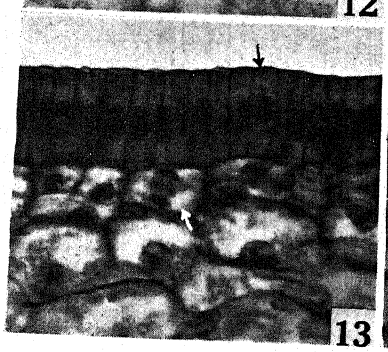

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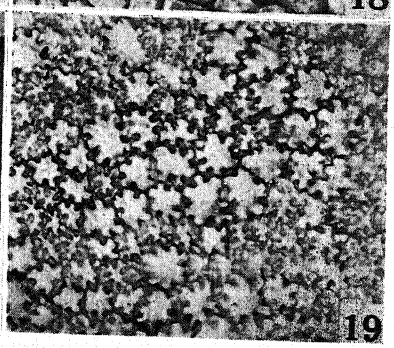

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show a tendency to develop lignified reticulations. Surrounding these is an extensive area of tissue with large air spaces and with irregularly shaped cells which possess abutting protuberances. This tissue is more compact immediately beneath the macrosclereid layers of the hilum (fig. 7). Throughout this zone the cells possess more or less pronounced wall thickenings which are strongly suggestive of the hypodermal origin and subsequent development of the osteosclereids. Their early development, prior to secondary wall formation, coincides with the early differentiation of the osteosclereids over the rest of the testa. Careful examination of sections of very young ovules shows that the cells immediately beneath the inner layer of macrosclereid initials of the hilum area are more meristematic and undergo greater proliferation than do the hypodermal initials which give rise to the osteosclereids elsewhere in the testa.

Growth of the macrosclereids.—As previously discussed, the macrosclereids are definable only as a protoderm, and the tissues which eventually form the rest of the testa, except for the ovule bundle, are ground meristem (see Foster, 1942, for classification of meristems). This condition prevails until the embryo sac is well enlarged and the proembryo is well formed. By the time the ovule is 1 mm. high the protoderm cells of the strophiole, of the top or dome of the ovule, and also those of the micropylar region have undergone some elongation as previously described (fig. 1 and 9). However, in the lateral sides of the ovule, the protoderm cells remain relatively unchanged. Elongation of the macrosclereids progresses from the regions where it is first evident; but until the process is clearly evident in the lateral sides of the ovule there is little evidence of the differentiation of the double row of macrosclereids in the hilum area. At no time, however, up to and including full maturity of the ripe seed, do the macrosclereids of the lateral walls elongate as much as those of the strophiolar region. There are no periclinal divisions, even in the strophiolar macrosclereids, as shown by Zimmerman (1936) for other species and genera. However, anticlinal divisions are numerous during early elongation of the macrosclereids (fig. 11–16).

Although anticlinal divisions continue at a remarkable rate during the early enlargement of the ovule, and up to sieve size one, cell division in this layer virtually ceases with the inception of secondary wall formation. When the fluted thickenings of the secondary walls appear (generally about sieve size one or two) there is little evidence of cytokenesis. It is worth while to call attention to characteristics previously described (Reeve, 1946a) in order to clarify this point. In different peas passing through sieve size one there is some difference in degree of tissue maturation. This is true for any given sieve size. Hence, the youngest peas of size one may contain macrosclereids in the lateral walls, and particularly in the hilum, which still show occasional cell divisions. In histologically older portions of the testa in these same peas, such regions as the strophiole will then contain macrosclereids showing early stages of secondary wall formation. Older peas of the same sieve size may show secondary wall formation beginning even in the youngest portions of the testa. Secondary wall development in the macrosclereids, with reference to the entire testa, follows a regional pattern which parallels the sequence of cell elongation, but not to such a marked degree.

Since the rapid rate of cell division ceases in the macrosclereids long before the ovule has reached full size, it is obvious that there must be some mechanism by which the testa also continues to enlarge. It may be difficult to conceive of an appreciable cell enlargement effecting an increase in the surface area of the testa occurring when the macrosclereids have developed as much secondary wall thickening as is evident during growth of commercial sieve sizes. However, this undoubtedly occurs (carefully compare fig. 17–19 and 21–24 for earlier stages of this growth).

When the macrosclereid has approached nearly its maximum length, at which time secondary wall thickenings have already begun (as in sieve size one), its greatest diameter may be only one third that of a comparable located macrosclereid in an ovule of full size or in the ripe condition (as in size six or seven). During growth from sieve size one to maturity at sizes five, six, or seven, and depending upon how nearly spherical in shape the ovule remains, the surface area of the testa may increase from two to three fold. Before there is any evidence of secondary wall formation, *i.e.*, the macrosclereid walls in fresh sections are isotropic, and when anticlinal divisions occasionally occur only in the "youngest" parts of the testa, as in an ovule of 4 mm. height, the surface area of the testa is only about one-tenth that of a full sized, nearly ripe seed (size six or seven).

The circumferential growth of the testa by cell enlargement is also expressed by characteristics of successive stages in the differentiation and growth of the parenchymatous tissues. Just before second-

Fig. 8–19.—Fig. 8. Section from wall of ovule about 1 mm. high and showing differentiation of protoderm. ×480.—Fig. 9. Section from strophiole of ovule about 1 mm. high. ×480.—Fig. 10. Higher magnification (×250) of part of strophiole shown in fig. 3; note the periclinal activity beneath the macrosclereids.—Fig. 11–16. Stages in elongation of macrosclereids in early differentiation, also showing anticlinal and periclinal cell divisions in subepidermal cells before differentiation of hypodermis or osteosclereids. ×480.—Fig. 11. Ovule slightly over 1 mm. high—Fig. 12, 13. Ovule about 2½ mm. high.—Fig. 14, 15. Ovule 3 mm. high.—Fig. 16. Ovule about 4 mm. high.—Fig. 17–19. Transsections of macrosclereids at different ages. ×480.—Fig. 17. From ovule about 4 mm. high; cells on left cut through distal ends.—Fig. 18. From ovule of sieve size 1; cells cut in plane just below middle.—Fig. 19. From ovule of sieve size 1; section through upper half of macrosclereids, showing early development of fluted thickenings.

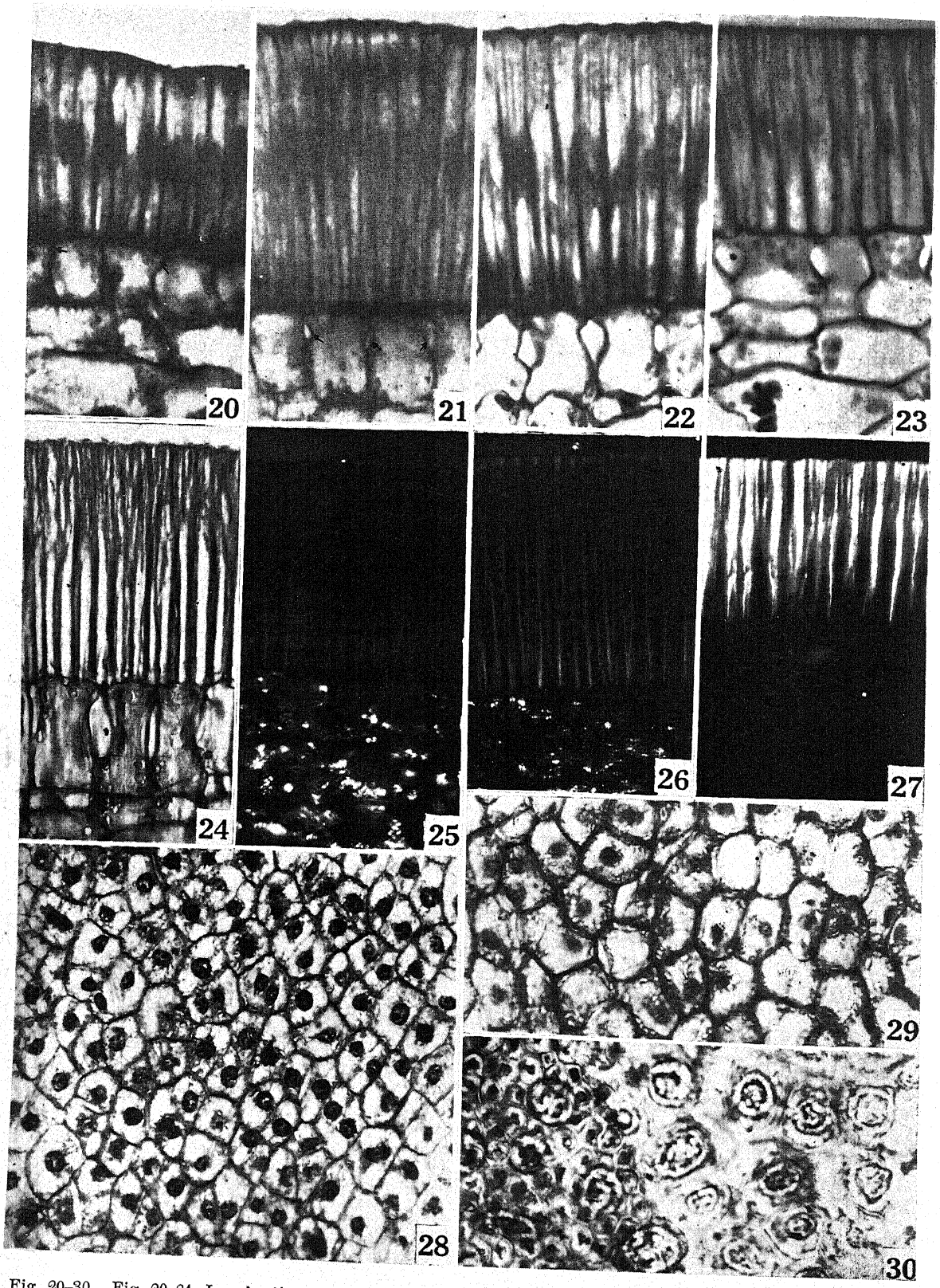


Fig. 20–30.—Fig. 20–24. Longisections of macro- and osteosclereids in stages following cessation of mitoses; arrows indicate early stages of intercellular space development in osteosclereid layer. ×480.—Fig. 20, 21. Younger and older stages, respectively, from ovule about sieve size 1; fig. 21 from top of ovule.—Fig. 22, 23. Younger stages found in

ary wall formation in the macrosclereids, the parenchyma cells have undergone greater enlargement in circumferential directions than in radial directions, but intercellular spaces have only begun to form. In the range of growth as expressed by commercial sieve sizes, when secondary wall thickening in the macrosclereids is greatly increasing, the parenchyma cells undergo even greater expansion in the circumferential directions of the testa and also develop large intercellular spaces which increase the volume of this tissue.

Obviously, one cannot evade the controversy of *gliding* versus *intrusive* growth. Either of these two theories taken alone is wholly insufficient to explain the growth phenomena described here. With reference to the macrosclereids, it is quite apparent that a certain amount of "gliding" of the abutting macrosclereid walls necessarily must occur in order to accommodate the tendency for one enlarging cell to "intrude" upon the space of its neighbors. Such "gliding" may be more pronounced in the macrosclereids of the hilum region where the tapering ends of the two layers of these cells overlap. It is remarkable that this sort and amount of growth occurs during stages of development in which there are occurring additive changes of cell wall materials characteristic of maturity, and which are usually considered to occur mainly after a cell has reached full size. However, there is no contradiction in the actual facts. Previous studies (Reeve, 1946a) on cell wall composition and the "encrusting substances" of the middle lamellae, as well as the stages in histogenesis described here, demonstrate that both the cell wall and the intercellular substances must remain in a relatively plastic condition until the tissues become rigid at maturity. The relationship of cell wall composition and intercellular substances, as physically influencing cell and tissue growth, constitutes a subject too frequently neglected and too little understood in modern developmental plant anatomy.

As previously mentioned (Reeve, 1946a), it is apparent that the mature macrosclereids shorten in length without any noticeable change in diameter when the tissues dry during the ripening of the seed. This shrinkage may result in a ten to twenty per cent decrease in length. It is accompanied by an apparent increase in brilliance of anisotropy which is suggestive of either increased crystallinity or some comparable change in cellulosic orientation. Berkley and Kerr (1946) have reported a comparable situation in the drying of cotton hairs. They found that hairs from developing bolls possessed considerably plasticity, showing little evidence of crystallinity when wet, and become increasingly crystalline when dried, stretched wet, and when stretched to the breaking point. They have suggested the presence and relationship of water molecules to the cellulosic matrix of the cells in the living and growing condition. A similar sort of relationship of water to the structural elasticity of the pentosan-cellulosic matrix of the secondary wall thickenings in the macrosclereids of peas could readily explain the mechanism for the decreased length upon drying, and also the apparent change in optical properties as described above.

Growth of the osteosclereids.—Differentiation of the osteosclereids proceeds in a regional pattern over the testa which parallels that described for the macrosclereids. However, the different stages of cell growth, and particularly those of early differentiation, lag considerably by comparison with the growth of the macrosclereids. There is no definable hypodermal layer at the time the macrosclereids of the strophiole are well elongated (fig. 10). Until the ovule is about 4 mm. high (nearly sieve size one), when divisions in the macrosclereids have virtually ceased, both periclinal and anticlinal divisions have continued in the cells from which the osteosclereids eventually develop. In the early stages when the protoderm cells have only begun to elongate, those cells immediately beneath them are similar in structure and activity to the rest of the ground meristem (fig. 8). When the cells of the ground meristem begin to enlarge, but before they contain any appreciable amount of starch, it is obvious that meristematic activity in tissues underlying the protoderm becomes localized to the region from which the hypodermis is to differentiate (fig. 11–16).

Underlying the macrosclereids of the strophiole the rate of periclinal cell divisions exceeds the rate of anticlinal divisions. Consequently this area assumes characteristics reminiscent of rib-meristem (Chap. 3 in Foster, 1942). This is clearly defined in a young ovule about 2 or 3 mm. high (fig. 10), but it appears to only a slight degree or is lacking in the lateral sides or elsewhere in the testa of the ovule. By the time the parenchyma cells of the testa have enlarged considerably and contain numerous starch grains, periclinal activity in the subepidermal cells diminishes and most of the divisions

ovules of sieve size two; fig. 22 from top of ovule. The fine dark line at the distal ends of the macrosclereids are loci of the fluted thickenings of the secondary walls in very early stages of formation.—Fig. 24. Later stage from top of ovule of sieve size two; note the more extensive development of the fluted thickenings of the macrosclereids and their twisted appearance in the distal portions of the cells.—Fig. 25, 27. Successive stages of macrosclereids viewed between crossed nicols. ×480.—Fig. 25. Comparable in age to fig. 21; note faint anisotropy in basal portions of macrosclereids.—Fig. 26. Comparable in age to fig. 22.—Fig. 27. Comparable in age to fig. 24, but from lateral side of ovule.—Fig. 28–30. Trans-sections of osteosclereids in successive stages of development. ×480.—Fig. 28. From ovule nearly 4 mm. high (compare fig. 16); note starch granules and cell lineages of osteosclereid initials.—Fig. 29. From ovule nearly sieve size one.—Fig. 30. From ovule of sieve size three or four; the plane of focus is at the level of the constricted equatorial regions of the radial walls, hence surrounding intercellular space is interconnecting and ends of osteosclereids are slightly out of focus. Smaller cells on left are basal views of macrosclereids.

occur in anticlinal planes. Further cell enlargement then follows (fig. 14–16, and 20). The hypodermis is then well defined and the cells contain some starch grains. A few starch granules persist even after these cells have assumed their characteristic shape as osteosclereids (fig. 22–24).

Thus, the ontogeny of the osteosclereids differs from that of the macrosclereids which have their origin from a well defined protoderm and at no time contain any readily visible starch grains. In addition, the mature macrosclereids form a compact tissue in contrast to the osteosclereids which become separated for a greater part of their lengths along their radial walls (fig. 24). Actually the intercellular spaces of the osteosclereids are all interconnecting, as can be seen from the plane of focus shown in fig. 30.

The development of this intercellular space is unique and it appears to be directly related to the differential rates of circumferential growth of the two tissues over the testa. When the ovule is 2 or 3 mm. high the young osteosclereids appear as vacuolate, more or less cubical cells which soon elongate (fig. 16 and 20). Upon further enlargement, after anticlinal divisions have ceased, these cells become more uniform in size and appearance (fig. 27 and 28). By this time further cell growth becomes restricted to the end wall regions of the osteosclereids so that the ends of these cells undergo greater tangential expansion than do the equatorial regions. Thus, it is clearly evident that the radial walls become separated as a result of differential stresses of growth (fig. 20–24).

When stained with methylene blue chloride, or when treated with chemical reagents (Kerr and Bailey, 1934; Reeve, 1946a), the intercellular materials in the end wall regions of the osteosclereids (before radial wall separation) appear more pronounced and probably are of somewhat different chemical composition from the middle lamellae substances of the radial walls. In addition, the walls of the radial sides of these cells mature more rapidly than do the walls at the ends of the osteosclereids. They develop the characteristic secondary wall thickenings of the constricted portions of these cells, and thus the osteosclereid becomes less plastic in its radial and constricted middle than at either of its ends.

Composition and properties of the sclereid walls during early differentiation.—*The primary wall.*—Histochemical tests indicate that the walls of young actively dividing and elongating macrosclereids are composed of a pectic-cellulosic complex. It seems reasonable that the pectic materials are present in the interstices of the cellulose matrix as well as in the middle lamellae. When subjected to the hydrocellulose test with dilute acid and iodine solution (Rawlins, 1933) there is a slight swelling and a subsequent diffusion of amyloid blue wall substance. The same is true of the young osteosclereids.

Applications of Bial's test and other tests previously reported for pentosans (Reeve, 1946a; Militzer, 1946) give no indication of the presence of structural pentosans. With Bial's reagent used as a pentosan test, the middle lamellae develop a red color. This may not be a true color reaction. The structural characteristics of the cell are rapidly destroyed by the acid. However, solutions of the reagent made with an increase in ferric chloride content gave positive results for the presence of pectins in both middle lamellae and primary walls.

That the middle lamellae of both macro- and osteosclereids in this early stage of development possess a high ratio of pectic materials can easily be determined by treatment of fresh sections with extracting reagents. Using either the technique of Kerr and Bailey (1934) for cambium, or by treatment with dilute ammonium oxalate or with one per cent oxalic acid (Norman, 1937; Reeve, 1946a) sufficient pectic material is extracted so that slight pressure on the cover glass over the section will readily separate the cells. As the cells mature and begin to develop secondary walls, treatment by the mild reagents does not so readily result in cell separation. The more resistant substances present are arbitrarily referred to as "encrusting substances."

Tissues which contain osteosclereids in a stage of development just prior to radial wall separation, when treated alternately with these mild reagents, show, by the greater resistance of the end wall regions to the reagents, that the middle lamellae materials between the radial walls are structurally weak. The results of tests applied to sections representing progressively older stages of development indicate that deposition of "encrusting substances" is increasing at the end wall regions during growth. It naturally fellows that when full size is reached, and further cell "growth" is limited to additive changes in the materials of the secondary wall and the middle lamellae (as in the larger sieve sizes), plasticity rapidly decreases.

In stages in which the secondary wall thickenings are pronounced, but before full size is reached (as in sizes 2, 3, or 4), attempts to differentiate the middle lamellae are difficult because of the incorporation of similar material into the adjacent walls. Thus, with aqueous methylene blue chloride (1:10,000), the more deeply violet staining of the middle lamellae, in the basal portions of the macrosclereids and the distal portions of the osteosclereids, is not sharply defined from the lighter blue of the walls because the deeper color extends into the walls.

Early formation of the secondary wall.—In fresh sections the primary walls of both macro- and osteosclereids are isotropic. The formation of characteristic fluted wall thickenings of both cells follows immediately a general uniform thickening of wall material which, in the macrosclereids, continues mainly in the basal parts of the cells. However, just prior to any visible indication of secondary wall formation, faint anisotropy may be seen

in the macrosclereids at the distal and basal portions of their radial walls (fig. 25, 26). This suggests that there is a beginning formation of microfibrillar structure and orientation as has been described for the mature cells (Reeve, 1946a). However, when cells of this stage of development are treated chemically, swelling is too severe to permit any accurate observation of fibrillar structure. A closely similar situation is also present in the radial walls of young osteosclereids.

In an ovule about 3 mm. high the macrosclereids of the strophiole show a slight development of the characteristic fluted thickenings in their distal ends. At slightly later stages of ovule development, the macrosclereids in the lateral sides of the ovules present the same structure in their distal ends (compare fig. 23 and 26). As these thickenings develop downward along the radial walls of the macrosclereids, similar thickenings in the radial walls of the osteosclereids become evident. When the latter are carefully examined between crossed nicols, and the sections rotated, it is evident that the end walls of the osteosclereids have remained isotropic and are without such thickenings.

The faint anisotropy previously mentioned appears independently of the beginning of these fluted thickenings which are responsible for most of the anisotropy seen in the walls of older cells (compare carefully fig. 25–27). Since the faint anisotropy is present before there is any other visible evidence of secondary wall formation, there appear to be no sharp distinctions between primary and secondary wall layers. This suggests that the cellulosic matrix may undergo a transition in crystallite orientation along the inner surface of the wall. This transition may be influenced by cell elongation, as one might infer from the investigations of Berkley and Kerr (1946) on cotton.

Whatever the nature of the transition may be, suitable explanations may be found in the structural conditions necessary for either "true birefringence" or "form birefringence" (Chamot and Mason, 1938). Should true birefringence cause the faint anisotropy, it is evident that the cellulosic matrix has undergone a transition in crystallite orientation, perhaps along the inner surface of the walls. On the other hand, form birefringence, as would be caused by the addition of new particles of wall substance laid down in rows corresponding, perhaps, to the loci of the fluted thickenings, would not necessarily involve crystallite orientation. However, in this second case, a transition in wall structure would also be apparent.

The nature of anisotropy in most materials can be determined by mounting in media of different refractive indices and making the proper tests. However, the structural nature of these cells at this stage and at older stages of development, and the difficulty of obtaining sections suitable to the purpose, constitute barriers to any accurate observations from which conclusive evidence can be obtained. The thickenings, even in early stages of appearance in the macrosclereids, twist irregularly at about a twenty degree pitch along the long axes of the cells. Consequently, there is too much overlapping of adjacent walls, even in 5 μ sections, to permit critical observation. Naturally, evidence was obtained that some form birefringence exists, and along with true birefringence contributes to the anisotropy of the secondary wall thickenings.

Discussion.—Although the integumentary structures of angiosperm seeds have been widely investigated (Pammel, 1899; Netolitzky, 1926; Zimmerman, 1936), details as to their histogenetic development have been neglected. Reeves (1936a, 1936b) has described the comparative anatomy of the seeds for a number of genera in the Malvaceae, but presents little information regarding the histogenetic development of the integumentary tissues. Reeves also reported results of several histochemical tests but used no reagent, such as Bial's, which would differentiate between lignin and pentosans in the cell walls. In the author's opinion, the phloroglucinol reagent is not reliable for this purpose.

Pammel (1899) and Zimmerman (1936) have compared various species of the Leguminosae as to seed structure and function, and Zimmerman has further considered the development and significance of the strophiole. In general, the histogenetic origins and development of the macro- and osteosclereids, as presented here for *Pisum sativum*, appear to be common to the other genera of the Leguminosae. However, the seeds of *Pisum* are structurally less specialized than are those of *Melilotus, Cicer,* and *Dolichos,* for example. Obvious differences occur in the hilum region, the structural development of the caruncle, the arillus, and the strophiole. In addition, the subcuticular caps of the macrosclereids are quite pronounced in some genera, as in *Melilotus,* and are absent in others, as in *Pisum.*

In *Cicer* the macrosclereids differentiate into alternating series of longer and shorter cells, presenting a structure somewhat analogous to the motor cells in the epidermises of certain grass leaves (Winton and Winton, 1935). At maturity of the seed these series form the ridges which are characteristic of the seed coats of *Cicer.* However, the distinction between wrinkled and smooth-seeded varieties in *Pisum* is not due to such a structural development, and appears to be related to some characteristic behavior of the integumentary tissues and their contents upon drying. In the main structural specializations of shape and cell wall development, the macro- and osteosclereids of the more common species in the genera *Canavalia, Dolichos, Lathyrus, Lens, Lupinus, Phaseolus, Pisum,* and *Vicia* are quite similar, and differences occur mainly in such regional specializations of structure as mentioned above.

The significance of cell wall structure to texture previously has been mentioned. The relationship is closely similar to those maturity changes in tensile strength and cell wall development in the cotton

hair, subjects which have been widely investigated in applied research. Although varietal comparisons of peas and other legumes have not been made histologically for the purposes of relating texture to maturity change and cell wall development, there are sufficient unanswered problems of texture in food research to indicate the need for more thorough histological data. It should be possible to approach at least partial solution to these problems through genetical development of new varieties possessing more suitable characteristics. In addition, more exact methods for measuring texture by mechanical penetration are required. Such methods, based upon histological information, should be of aid to the geneticist.

SUMMARY

The comparative histogenesis of the macrosclereids and osteosclereids in the testa of *Pisum sativum* has been presented. The macrosclereids are derived from a well defined protoderm in the young ovule. They follow a regional pattern of differentiation over the testa, first becoming extremely elongated in the strophiole before appreciably elongating in the lateral walls of the ovule. Early circumferential growth of the ovule occurs while the macrosclereids undergo rapid anticlinal divisions. Later growth occurs during further elongation and also enlargement of the macrosclereids in tangential directions.

The osteosclereids remain undifferentiated through the earlier stages of macrosclereid development. Rapid anticlinal and periclinal divisions precede initiation of the hypodermal layer. Cessation of periclinal divisions results in differentiation of the young osteosclereids which follow a pattern of regional development over the testa paralleling that of the macrosclereids.

The characteristic shape of the osteosclereids and their intercellular spaces are related to (1) diminution of anticlinal divisions after the hypodermal layer is formed, and (2) separation of the radial walls of the cells and continued enlargement of their extremities.

Certain features of tissue differentiation are related to characteristic specialization of the cell wall, its changes in composition, and cellulosic orientation or crystallinity. The relationships of sclereid growth and development to "gliding" and "intrusive" growth are briefly discussed.

The primary walls and middle lamellae of the sclereids are isotropic. Anisotropy is apparent before any other visible evidence of a definable secondary wall structure or layer. This suggests a transition from the primary wall condition to that of secondary wall formation.

The characteristic thickenings of the macrosclereids are first evident in the distal portions of the radial walls. They appear at the same time or just following general and uniform thickenings of the walls in the basal portions of the cells. Secondary wall thickenings are initiated in the equatorial areas of the radial walls of the osteosclereids.

Comparisons of the sclereids and structural specializations in *Pisum* and other Leguminosae have been made. The relationships of histological structure and cell wall composition to tissue texture and its significance to applied fields of research also are briefly discussed.

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GENERAL SECTION

(Abstracts of this Section are arranged alphabetically according to author)

Artschwager, Ernst, Bureau of Plant Industry, U. S. Dept. of Agriculture. *Pollen degeneration in male-sterile sugar beets with special reference to the tapetal plasmodium.*—Pollen abortion in completely male-sterile beets is associated either with a periplasmodium or a cellular tapetum. Both types may occur within a flower cluster but not within the limits of a single flower. The presence of a plasmodium somewhat delays pollen abortion, but where the tapetum remains cellular some microspores are destroyed while still in the tetrad. In both types, degeneration is accomplished long before the endothecium has matured. In semi-male-sterile beets a plasmodium never develops although the cells of the tapetum may hypertrophy greatly and fill the anther cavity. Such hypertrophied tapetal cells have large nuclei and vacuoles like those in the periplasmodium. The cytology of the young plasmodium suggest hypermetabolic activity, but not for the benefit of the developing microspores. It seems to develop as a structural continuity even though it makes pseudopodium-like incursions into the anther cavity. After the periplasmodium has attained maximum development, it begins to degenerate. The cytoplasm becomes coarsely alveolar and then stringy; the nuclei retain a certain degree of organization but they too finally degenerate. With the complete degeneration of the plasmodium the microspores are also destroyed. The anther cavity becomes empty except for a strand of blackened matter which occasionally still shows evidence of cellular structure.

Ball, Ernest, University of North Carolina, Raleigh, N. C. *Segmentation of the shoot apex.*—Two longitudinal cuts at right angles to each other were made in the shoot apex of *Lupinus albus* L. Subsequent to the operation the four pieces of the original meristem did not grow together, but each piece grew separately and usually regenerated a normal shoot apex. Subjacent to the cuts, each new meristem produced new provascular strands in its leaf primordia and thus completed the ring of provascular tissue seen in transverse sections. Each of these new shoot apices grew normally and produced a normal shoot. These new provascular tissues were formed acropetally by the regenerated shoot apices and connected below with the original single ring of vascular tissues. This behavior demonstrated the following things: (a) This is an example of plant regeneration in the zoological sense of the term; most plant regeneration is a dedifferentiation process. (b) No central group of initial cells in the original shoot apex is essential to the function of that meristem; this operation destroyed the central cells. The lateral cells of the shoot apex are able to function as initials and to grow and produce all parts of a normal shoot. This constitutes experimental proof that the various regions of the meristem do not have specific unalterable destinies. (c) The production of four shoots after the dividing of the original meristem into four parts constitutes further evidence that the shoot apex normally produces the subjacent parts (epidermis, cortex, provascular tissues, pith) of the shoot by its own independent activities; an alteration in the meristem has changed the nature of the shoot produced by the meristem. This is experimental evidence that the shoot apex produces the tissues of the shoot independent of control by the mature subjacent shoot.

Berger, C. A., Fordham University, New York, N. Y. *Cytological effects of combined treatments with colchicine and naphthalene-acetic acid.*—In *Allium* root meristem colchicine prevents spindle formation, causes diplochromosomes which divide and revert to form tetraploid cells. It does not affect resting cells nor cause tetrachromosomes. Naphthalene-acetic acid does not affect apical meristem but stimulates division in the region of elongation; normal diploid divisions in the pericycle and tetraploid divisions with 16 tetrachromosomes in the large $4n$ cortical cells. There is a 24 hour lag between treatment and effect. Three combination treatments were made. Colchicine following naphthalene-acetic acid caused very many $2n$ c-mitoses

in the meristem and polyploid (4*n*, 8*n*, 16*n*, and 32*n*) c-mitoses in the cortical cells of the region of elongation. Naphthalene-acetic acid following colchicine gave the colchicine effect (c-mitosis) in the meristem and the naphthalene-acetic acid effect in the region of elongation—4*n* mitoses with tetrachromosomes but no polyploid c-mitoses. Treatment with a mixture of the two solutions gave results similar to those of colchicine following naphthalene-acetic acid. One striking difference resulting from this treatment was the presence of supercontracted diplochromosomes in which the length and the width were equal.

BLAKESLEE, A. F., S. SATINA, AND A. G. AVERY, Genetics Experiment Station, Smith College, Northampton, Mass. *Genetic evidence suggesting that egg cells in Datura may sometimes develop from the epidermal layer.*—It is currently believed that eggs and pollen in plants are derived from the second or subepidermal layer. Some genetic evidence is offered that this may not always be the case. Three 4*n*, 2*n* periclinal chimeras pollinated by a 4*n* dominant "Bronze" gene gave as expected some 3*n* Bronze offspring, presumably from fertilization of 1*n* eggs. In addition, however, there was the unexpected appearance of 4*n* Bronze offspring. Conceivably these latter might have come from fertilization of reduced eggs which had doubled their chromosome number, or more likely from fertilization of a 2*n* nucleus derived through reduction from 4*n* epidermal cells. In addition, two 2*n*, 4*n* chimeras crossed with a 2*n* Bronze gave as expected Bronze 3*n* seedlings but also a number of Bronze 2*n* offspring. These latter would seem explainable only by the fertilization of a 1*n* nucleus derived through reduction from an epidermal cell. The genetic evidence, therefore, points to the derivation of some egg cells from the epidermal layer in these cases unless the periclinal plants used in the crosses may have been mixochimeras as well as the types of periclinal chimera described. This we do not believe was the case. Histogenic study of course will be necessary before it can be considered established that the epidermal layer is capable of functioning as generative tissue.

BLASER, H. WESTON, AND JOHN EINSET, University of Washington, Seattle, Wash., and New York State Agric. Expt. Sta., Geneva, N. Y. *Leaf development in a periclinal chimera of "Spy" apple.*—The stem apex consists of a two-layered surface of diploid cells covering a tetraploid central portion. There is no uniform difference in cell size, nuclear size or number of nucleoli in the two zones. It is possible to identify only those cells in which chromosomes are in suitable condition for counting. In reconstructing the leaves from sections, the veins were plotted and tetraploid cells were assigned to their approximate positions. The petiole is largely tetraploid. The diploid portion of the "cortical area" varies from two to ten cells in width. The stipules contain tetraploid veins but surrounding parenchyma is diploid. In the blade, all principal veins and many smaller veins have been identified as tetraploid. Occasionally, a large cell, lateral and adjacent to a vein and isolated large cells imbedded in the mesophyll are also tetraploid. These may be "mother cells" of veins to be developed during later growth of the leaf. The parenchyma and epidermis are diploid. It is evident in this chimera that the development of the leaf follows a complex, branching pattern. The origin of the blade is from many ranks of "growth areas" which are webbed into a flat leafy structure. The major direction of initiation must be in line with the principal veins and is oblique rather than strictly lateral. Cross-sections which seem to show a linear "marginal meristem" usually illustrate the derivation of mesophyll cells independent of the associated tetraploid tissue.

BRITTON, M. E., Northwestern University, Evanston, Ill. *Freshwater algae from southwest Pacific islands.*—At infrequent intervals during the years 1945-46 opportunity was presented to study various algal habitats and to collect specimens over limited local areas near the coast at Milne Bay, New Guinea, on the south side of Biak in the Schouten Islands, and in Leyte Province, the Philippines. Examination of 180 samples of algae has resulted in a list of rather common cosmopolitan species. Among the more interesting finds are several new species of Chlorophyceae. *Draparnaldiopsis indica* Bharadwaja is reported from Palo, Leyte. This is the first record of this species since it was originally described from the type station at Benares, India, in 1933.

BROWN, W. V., Brown University, Providence, R. I. *A cytological study in the Paniceae.*—The basic chromosome number characteristic of the Paniceae is 9, and this basic number is found in 77 species and varieties of the genera *Digitaria, Leptoloma, Axonopus, Sacciolepis, Oplismenus, Echinochloa, Setaria, Cenchrus, Amphicarpum* and *Panicum.* In *Paspalum* and *Stenotaphrum,* however, the basic number is 10. In *Digitaria sanguinalis* an aneuploid series of plants was found with chromosome numbers intermediate between the tetraploid and hexaploid. In *Cenchrus* the chromosome number $2n = 34$, as found in *C. tribuloides,* has been derived, by the loss of one pair of chromosomes, from $2n = 36$, as found in *C. pauciflorus.* Various species of *Paspalum* are found to be diploid, tetraploid, hexaploid and 12-ploid while *P. praecox* has both diploid and tetraploid forms. Chromosome fragments of varying number were found in plants of *Panicum virgatum.* In *Panicum,* section Agrostoidea, six of the seven species were studied and all are diploid. In *Panicum* subgenus *Dicanthelium* the chromosome numbers of 46 species and varieties were determined. All are diploid except *P. clandestinum* and *P. boscili* var. *molle* which are tetraploid. *Axonopus affinis,* reported by another worker to have $2n = 80$ chromosomes, was found to have $2n = 54$ chromosomes in the material studied.

CARLSON, MARGERY C., Northwestern University, Evanston, Ill. *Plant hunting in El Salvador, C. A.*—About a thousand species of plants which were in bloom during the dry season were collected in January, February, and March, 1946, under the auspices of the Chicago Natural History Museum and Northwestern University. El Salvador is thickly populated and extensively cultivated and there are comparatively few areas of undisturbed natural vegetation. The most interesting plant communities were the cloud forests on the peaks of the volcanoes. Some of the unusual plants will be illustrated with kodachrome slides.

CROSS, GEORGE L., AND NORMAN H. BOKE, University of Oklahoma, Norman, Okla. *Floral development in Vinca rosea L.*—The present investigation employs the ontogenetic approach to the problem of floral interpretation and utilizes a plant in which apical meristem structure and leaf development are already known. Apices of mature plants of *Vinca rosea* produce two floral primordia in the axil of one member of each pair of decussate leaf primordia. The flowers are of unequal age, the youngest always being nearer the subtending leaf. The floral apex has a two-layered tunica. There appears to be no reason for regarding it as "essentially" different from a vegetative apex. All floral organs arise by periclinal divisions in the first two or three hypodermal layers. Sepal primordia arise very early. Other floral organs do not appear until the floral apex has enlarged and become broad and flat. Petal formation is followed closely by the initiation of stamens and carpels. Sepal, petal, and carpel primordia undergo a brief period of apical growth, and have a marginal meristem. Stamen primordia produce archesporial and tapetal cells from the region which might be expected to give rise to a sub-marginal meristem. Carpel primordia are leaf-like in early development. Ovules arise from the marginal meristem. The two structures which alternate with the carpels contain vascular tissue. Their morphological significance is not clear. In early stages, the flower of *Vinca rosea* is polypetalous. The corolla tube arises very late and is the product of intercalary growth. Development as a whole supports the classical interpretation of the flower.

DEAN, H. L., AND J. FISHER STANFIELD, State University of Iowa, Iowa City, Iowa, and Miami University, Oxford, Ohio. *The rate of pollen tube growth in Lychnis alba.*—Lychnis has been used in a number of experiments in which the rate of pollen tube growth was an important factor. However, it appears that no direct measurements have been made of the speed of pollen tube growth; the rate of growth having been approximated by inductive methods. In the present study female flowers of *Lychnis* were heavily hand pollinated; the flowers killed at regular intervals after pollination and thick (35–40 μ) longitudinal sections made of the ovaries. Pollen tubes were observed in the placental bridge between the ovary wall and the free central (at maturity of the flower) placenta. Since all pollen tubes entering the placenta must pass through this bridge their presence, numbers, and rate of growth could be determined.

DEVINE, VERONA, State University of Iowa, Iowa City, Iowa. *Floral abscission in Lychnis alba.*—Unpollinated pistillate flowers of *Lychnis alba* abscise in an unusual manner. Separation occurs, not by the dissolution of the middle lamella, but by the passive breaking of the cell wall at a right angle to it. No meristem or cork cambium is differentiated in the abscission zone. This region is first evident as a roundly wedge-shaped furrow encircling the apex of the pedicel. Separation begins in the epidermis and develops inwardly through the large vacuolated cortical and pith parenchyma cells. The cell contents remain apparently unchanged for a short time after the walls have been broken. The collapsing cortical cells draw the epidermis centripetally forming a pseudo-protective layer. Abscission of the flower is completed by the mechanical rupture of the vascular bundles. At the time of floral abscission there is an increase of lignified and ligno-suberized tissue at the base of the pedicel.

DITTMER, HOWARD J., University of New Mexico, Albuquerque, N. M. *A critical study of subterranean plant parts.*—Approximately twenty-five species of plants from fifteen different families were studied to learn the total extent of root hairs on roots in various plant groups and also to determine if subterranean plant parts may be used alone in the identification of plants. Results show that root hairs are not confined to a "region of absorption" nor are they "short-lived structures." Roots of all categories (including primary, secondary, tertiary, etc.) were microscopically examined and in all plants where *considerable* secondary thickening had not occurred, numerous root hairs were found covering the entire root system. Persistence of root hairs became evident on some plants where the epidermis was sloughing off but hairs were still intact on their cells. Characteristics which may prove helpful in the identification of subterranean plant parts include the presence of stolons and rhizomes, manner of branching at the nodes, and number of roots arising from the nodes and internodes. Shape of the root tip may vary considerably. The root cap of Bermuda grass comes to a sharp point while the root cap and meristem of the dwarf French Marigold is bulbous. Secondary thickening may be absent, or present only in the tap root of some annuals, while in others it may be present in all categories of roots. Many other characteristics of plants growing in the field, will be discussed, including some which may be modified by the environment.

DODD, JOHN D., Columbia University, New York, N. Y. *Three dimensional cell shapes of cambial initials in Pinus silvestris.*—A modification of the wax plate method was used to construct models of cambial initials from serial sections of young stem tissue of *Pinus silvestris*. Camera lucida drawings of succeeding sections of each cell were trans-

ferred to thin sheets of the base plate wax used in dentistry. The wax sections were separated by distances determined from the thickness of the sections and the magnification. Black thread was then used to connect corresponding vertices of the serial sections and thereby served the double purpose of forming a framework of the model and outlining its individual faces. Then small pieces of the wax were cut to fit the exterior surfaces of the model and fused into position with a hot needle. At the time of writing this abstract the data are not complete, but a few general deductions are warranted. The number of contacts per cell varies from less than 10 to more than 27. The average will probably be considerably more than 14. Cell size affects the number of contacts and, as is generally accepted, cell division temporarily reduces both the size of the cell and the number of contacts. However, the spacing of ray initials is a prominent factor in disturbing simple geometrical relationships and increasing the number of contacts on fusiform initials. The number of sides per face varies from 2 to 14 or more. Tangential faces separating daughter cells usually have the largest number of sides.

Doerpinghaus, S. Leonard, Genetics Experiment Station, Smith College, Northampton, Mass. *Relative rate of embryo development in ten species of Datura.*—A study of the time requirement for development of embryos of ten species of *Datura* from pollination to comparable morphological stages shows pronounced species differences. The four stages, pro-embryo, heart, torpedo, and mature embryo are found in all species. There is little if any difference in the sizes of comparable early stages in the ten species, but the time required to reach such stages differs. Thus, pro-embryos about 0.1 mm. in diameter have been dissected from *D. stramonium* in seven to ten days. Comparable stages in D. metel are found in fifteen to twenty-one days from pollination.

Doty, Maxwell S., Northwestern University, Evanston, Ill. *The vertical distribution of marine algae at Woods Hole, Mass.*—Investigation of a marigram of the tides of the Woods Hole area shows some critical factors similar to those described as of primary importance in controlling the vertical distribution of marine organisms along the Pacific coast. As a result, in the intertidal algal cover, a number of horizontally extensive zones are recognizable one above the other.

Eigsti, O. J., Northwestern University, Evanston, Ill. *Mitotic rates in diploid and tetraploid cells of pollen tubes of Polygonatum.*—A comparison of mitotic activity among the cells of diploid and tetraploid populations shows that the peak of mitosis is reached earlier in the diploid than the tetraploid. The generative cell of pollen tubes of *Polygonatum,* a diploid species *P. pubescens* (n-10) and a tetraploid *P. commutatum* (n-20), served for these studies. Numerous sample populations of two hundred tubes per sample, one hundred for each species, were used. Comparisons of the diploid *vs.* tetraploid were based on material cultivated *in vitro* and on the same slide. The indices of mitosis were obtained from slides fixed at three, four, five, and six hourly intervals after the planting of pollen in the media. At the time pollen is planted into the media the nucleus of the generative cell appears to be in an interphasic stage. An index of mitosis is derived from the ratio of one-celled pollen tubes to two-celled pollen tubes and this index is in turn used for comparisons of mitotic activity between representative populations of diploids and tetraploids. The increase in mitotic activity among diploid cells and tetraploid cells is correlated with increased rates of growth noticed among diploid populations of plants. From field observations and herbarium studies the diploid begins flowering about one month earlier than the tetraploid.

Foster, Adriance S., University of California, Berkeley, Calif. *Ontogeny of the foliar sclereids in Mouriria Huberi Cogn.*—In the mature lamina, the sclereids are thick-walled columnar cells which branch more or less profusely beneath each epidermal layer. As is true of the majority of spp. of *Mouriria* investigated, the sclereids are terminal in position: *i.e.,* they are restricted to the ultimate ends of the veinlets. In *M. Huberi* the sclereid initials arise deep within the tissue of the young lamina, and are well-defined from neighboring tissue elements by their larger nuclei and more highly vacuolated cytoplasm. A comparison of trans- and paradermal sections indicates (1) that the sclereid initials and the procambial strands originate from the same embryonic cell layer, and (2) that at the earliest detectable stage of differentiation, the sclereid initial lies at the terminus of a procambial strand. During its early development, the sclereid grows vertically or obliquely between the various cell layers, and ultimately ramifies beneath the young epidermis. Very frequently, the tips of the subepidermal branches extend between the radial epidermal walls. When its intercellular development has been completed, the sclereid forms a thick, laminated secondary wall which is pitted only in the region of attachment to the veinlet.

Glock, Waldo S., and R. A. Studhalter, Texas Technological College, Lubbock, Tex. *Classification and multiplicity of growth layers in the branches of trees at Lubbock, Texas.*—Before climate can be inferred from a study of annual growth layers in trees the ability to identify annual increments must be acquired and certain basic anatomical responses to physiological processes conditioned by ecologic factors must be investigated. Preliminary but necessary steps to the general problem of the relationship of tree growth and climate are a definition and description of growth-layer types and a determination of the exact time or times during a season when growth layers are formed. Thus far these steps have been concentrated chiefly on the branches of trees at Lubbock, Texas. A descriptive classification becomes a requirement because growth layers in the branches of trees are not necessarily

concentric circles as seen in cross section, or simple tapering cones in longitudinal extent. Actually, growth layers may have wide variation in definition, and in extent around the circumference and up and down the branch. They may be entire; they may be lenses, half-lenses, or merely arcs. It is not at all uncommon to find annual increments composed of multiple growth layers. Radial additions to a growth layer may occur in a rather complicated manner in respect to speed, time, and location. In order to obtain the exact time of xylem formation, three methods were used: natural frosts, artificial frosts, and measurements of tip growth. The results of the investigation to date indicate that each of the types of growth layers classified above may be present as multiples in annual increments. It remains to be determined how far such multiplicity extends from the branches down into the trunks.

GOLUB, SAMUEL J., Harvard University, Cambridge, Mass. *Studies in the development of the vegetative shoot of Equisetum arvense* L.—No recent attempt has been made to reconcile the whorled arrangement of leaves in *Equisetum* with the helically produced derivatives of the well-known apical cell in the shoot apex. The results of the present investigation on *Equisetum arvense* L. support the thesis that no direct correlation exists between this helical pattern and the later-appearing whorled pattern of leaves at the nodes. Instead, the incidence and number of leaf primordia in any whorl seem to be correlated with the number and position of procambial strands which develop continuously and acropetally up to and into the first primordial leaf whorl from the established vascular supply below. The shoot apex initially consists of a series of closely superposed nodes which become separated by a series of intercalary meristems, each of which originates in the apical meristem as the basal portion of the leaf whorl sheath, and continues its activity as a detached meristem which is responsible for the entire growth of the internode during the elongation of the latter. The primary vascular tissues first differentiate at the base of the third or fourth node, the protophloem slightly preceding the protoxylem. Both develop acropetally and basipetally until a union is established with the mature tissues below. Both protophloem and protoxylem are continuous across the nodes within the same procambial strand. The vascular strand is therefore regarded as a continuous and unitary structure in both node and internode, although the subsequent more massive development of vascular tissues at the node tends to obscure the original individual strands.

HEINIG, KATHERINE, Connecticut College, New London, Conn. *Some aspects of the floral morphology of the Thymeleaceae.*—In order to determine the exact nature of the petaloid scales or petals found in certain of the *Thymeleaceae,* the morphology and vascular anatomy of the flowers of representative species were studied. In some species these organs are absent. When they are present they are to be found inserted at the summit of the floral tube inside of and alternate with the sepal lobes. These petal-like organs may be membranous and vary from the minute up to structures equal to the sepals in size; or they may be thick and fleshy in which case they may be glandular. The vascular supply to each of these petaloid structures or petals is very much reduced and consists of two weakly developed strands which represent the lateral petal traces. A median petal trace is wanting. These traces are adnate to the fused lateral sepal traces throughout the length of the floral tube and become distinct from these sepal traces only after the two lateral sepal traces become separated from one another. The petal traces may extend out into the petals for some distance as in the fleshy and glandular types, or, as in some of the membranous forms, only into the base of the petal. In some species having markedly reduced petals the vascular traces to these organs are lacking. Evidence would seem to indicate that these petaloid scales are reduced or modified petals in which the reduction and disappearance of the vascular supply seem to have preceded that of the organ itself.

HIGINBOTHAM, NOE, AND E. L. POWERS, University of Notre Dame, Notre Dame, Ind. *Some quantitative data on the red pigment produced by the fungus Coccosporium.*—As a basis for elucidating the role in metabolism of some of the pigments in fungi a study was initiated of the effects of various factors on the production of an unknown red pigment by an isolate of *Coccosporium* sp. (?). Low temperatures favor the accumulation of the red pigment whereas high temperatures result in the formation of a brown substance of different spectral absorption. Total pigment increases with increasing sugar concentration—glucose or sucrose—up to approximately 0.6 M. Relative to oxidizable carbon, glucose favors color formation more than sucrose. The amount of pigment formed seems not to be directly dependent on mycelium dry weight. Extremes of hydrogen ion concentration *e.g.* pH 3.0 and 8.5, result in lesser amounts of pigments but pH 4.0, 5.0 and 7.0 are superior to pH 6.0. The data show that there is considerable variability in pigment production but indicate that it is largely a function of controllable factors and plays some part in the normal metabolism of the *Coccosporium* sp. studied.

HOLTZMAN, DORIS HARRISON, Columbia University, New York, N. Y. *Three-dimensional cell shape studies in the vegetative tip of Coleus.*—By means of a modification of the Duchartre-Lewis technique, stem tips of *Coleus blumei,* Benth. were studied in cross and longitudinal sections and the average number of contacts determined. The stem tip of *Coleus* may be divided into two types of meristematic cells; those which are densely protoplasmic and those which are vacuolated. Complete data have not been assembled as yet. Preliminary tabulations indicate that the average number of contacts for all the cells was somewhat less

than 14, the small protoplasmic cells having a slightly lower average than the larger vacuolated cells. Such data were also recorded for the axillary buds and, as was expected, the results were similar. Further studies are being made on the types of faces and their frequency of occurrence in these two regions of the apical tip and axillary bud. Thus far the faces range from quadrilaterals to octagons.

HULBARY, ROBERT L., Department of Botany, State University of Iowa, Iowa City, Iowa. *Three-dimensional cell shape in the root tuber of Asparagus and the leaf parenchyma of Rhoeo.*—It has been suggested by Meeuse that the cortical parenchyma of the root tubers of *Asparagus Sprengeri* Regel and the hypodermal leaf parenchyma of *Rhoeo discolor* Hance are tissues in which the ideal cell shape, Kelvin's orthic tetrakaidecahedron, is of common occurrence. The three-dimensional shape of 100 cells from each of these tissues was determined. The number of faces per cell varied from 10 to 19 with an average of 14.20 in the root tuber and from 11 to 21 with an average of 14.24 in the leaf parenchyma. The kinds of faces on the 200 cells studied varied from triangles to octagons. Ninety-five per cent of the faces were quadrilateral, pentagonal or hexagonal. Pentagonal faces predominated in the root parenchyma, and hexagonal faces predominated slightly in the leaf parenchyma. The combination of faces typical of the orthic 14-hedron,—6Q and 8Hx—was encountered on 2 cells in the leaf parenchyma of *Rhoeo*. There were, however, several other configurations more characteristic of the tissue. Eight cells were found with, 4Q, 4P, 4Hx; 7 cells had, 3Q, 6P, 4Hx; and 8 cells had the combination, 4Q, 4P, 5Hx. These same three combinations were found to be characteristic of the root tuber tissue, and they occurred on 6,6, and 5 cells respectively. In the 200 cells studied 73 different combinations were recorded with 59 in the root parenchyma and 40 in the leaf parenchyma. Thirty-two combinations occurred on more than one cell. Of the 32 repeated combinations 23 were common to both tissues. Though no pattern stands out as one from which all others may be derived, there is evidence of more uniformity of cell shape within these two tissues than in any parenchymatous tissue studied previously.

JACOBS, WILLIAM P., Harvard University, Cambridge, Mass. *The developmental morphology of the intercalary meristem in the gynophore of the peanut plant.*—Mitoses showed a longitudinal distribution curve which was skewed toward the apex. The meristem was more than 5 mm. long. The number of mitoses per unit area was greater in the pith than in the cortex, but the longitudinal extent of active cell-divisions was less. The three-dimensional shape of the meristem is that of a cylinder with a thick plate across the center. The cylinder is composed of the meristematic tissue in the cortex which extends from 1 mm. to more than 6.3 mm. from the apex. Across the central "cavity" of this cylinder at approximately the 3 mm. level extends the region of maximal mitoses in the pith. Because the mitotic distribution in the pith comes to a sharper maximum than does that in the cortex, the pith meristem would take the form of a diaphragm-like band in a three-dimensional view. The region of maximal mitoses for the gynophore as a whole coincided with the region of maximal elongation of the gynophore. There was a correlation between the absence of pitted or reticulate xylem elements and the occurrence of rapid elongation. In this detailed study of an intercalary meristem, it is of particular interest that annular (or spiral) xylem elements and sieve tubes traversed the whole length of the actively dividing meristem.

JEFFREY, EDWARD C., Biological Laboratories, Harvard University, Cambridge, Mass. *Hormones in relation to reproduction and mutation.*—It is quite generally assumed in plants when, if after pollination and the germination of the pollen on the stigma of the ovary, seeds are formed, that these are the result of a sexual union; in other words, that a fusion of male and female nuclei has taken place. It is the purpose of the present communication to show that this is often not the case. Contrary to the received conception, there is no union of sexual nuclei as in a normal reproductive act. On the contrary, although in fact the deposition of pollen on the stigma is a prerequisite to the production of seed, no pollen-tube descends the style to effect fertilization. The production of seed is clearly due to the action of hormones operating at a distance from the stigma and through the tissues of the style, the ovary and the ovules. Ordinarily, as is shown by extended observations on a wide range of objects, the unfertilized egg, bearing the haploid or gametic number of chromosomes, as a result of hormonic stimulus develops the diploid or somatic number. Thus an embryo is formed without fertilization. The consequences of this situation are remarkable. Since there is no sexual union in the ordinary sense, the variability resulting from the union of male and female elements is absent. The germinating seeds as a consequence give rise to a uniform progeny; in other words "breed true" and are typical mutants or "elementary species." This situation is well illustrated by the genera *Trillium* and *Tradescantia* but a preliminary investigation has revealed its wide occurrence in higher organisms. This remarkable situation appears to have an extensive theoretical and practical significance.

JOHNSON, M. A., Rutgers University, New Brunswick, N. J. *On the occurrence of xylem associated with the strands of internal phloem in the tomato.*—Vessels, consisting of short, broad segments conspicuously marked with scalariform pits, were found in fourteen foot stems of the Michigan Forcing variety of tomato. Vessels were generally differentiated in from one to six strands of the internal phloem. They were not continuous throughout the stem but frequently died out for several internodes only to be resumed again to

extend through as many as twenty internodes. Instances were found where they were differentiated in the peduncle of fruit clusters.

KING, GLADYS S., Bureau of Agricultural and Industrial Chemistry, New Orleans, La. *Study of citrus vesicle constituents stained by fat-staining reagents.*—The "fatty" or lipid constituents of citrus fruits are of importance in relation to quality and deterioration of the canned juices and other products. Davis (Amer. Jour. Bot. 19: 101, 1932) described discrete oil deposits or glands in the vesicles which were defined by reaction with osmic acid. It was not possible to duplicate these observations but deposits and tissue structures stained by osmic acid and a number of oil soluble dyes are found peripherally in the vesicles and in spaces between them. These structures contain suberin which was identified by microchemical tests and isolation of phellonic acid. Fat soluble stains serve to define intercellular plastids in the vesicle tissues. The plastids swell on addition of acetone and separate into the acetone soluble contents and acetone insoluble plastid wall substances. Quantities of plastids collected by electrodialysis have been separated into these two fractions, the waxy wall substance and an orange colored oil from which crystals of carotenoids separated.

LANGDON, LADEMA M., Goucher College, Baltimore, Md. *Beeches of the Antarctic. Significant features of their comparative morphology.*—While the distribution and general ecology of the known species of southern beeches, as also their taxonomic problems, have been dealt with by several investigators, including W. J. Hooker, Blume, Oerstedt, Bentham and Hooker, Reiche, Dusen, Elwes and Henry, Cockayne, and others, very little—other than of their external taxonomic characters—is known about the morphology of these important members of the beech series; even less, in fact, than with respect to the morphology of other sections of the Fagaceae. Data from the present comparative survey of the structural features of species of *Nothofagus* reveal: (a) distinctive histological and anatomical features of their leaves; (b) details of variation in inflorescence and floral structure in species of the genus, in particular that of the pistillate flower-heads; (c) internal morphological characters in flowers and fruits sufficiently constant in nature to be of diagnostic value in a definition of the genus. The beeches from the Netherlands New Guinea—concerning which there has been some question as to position in the Fagacean series—have been found to exhibit a combination of morphological characters, in their foliage and reproductive structures, which are distinctly those of *Nothofagus;* although in certain respects sufficiently unlike the more typical members of this genus to justify their reference to a new section of the evergreen beeches.

LARUE, CARL D., University of Michigan, Ann Arbor, Mich. *Root hairs on stems and leaves.*—Very young hypocotyls of seedlings from which the roots have been removed produce outgrowths identical with root hairs. These structures form also on petioles and blades of excised cotyledons. In no instance have they been found on normal, unwounded seedlings. They are produced not only by epidermal cells, but also by parenchymatous cells on the cut surfaces. They do not develop near the collet and will not form on the lower hypocotyl of the tomato, although other species do not show this restriction. Both simple and branched hairs are found. These hairs bear no resemblance to ordinary hairs of stems and leaves. When exposed to dry air they collapse as quickly as normal root hairs, while the stem and leaf hairs are unaffected. The rate of development varies, but in tomato their formation may occur within 48 hours after excision. They occur on tomato, radish, cabbage, kohlrabi and collards, but not on castor bean, squash, flax, bean or any monocotyledonous plant. They are not dependent on development of adventitious roots inside the excised organs, as was suspected, but may arise before any sign of primordia of adventitious roots may be seen. The cotyledons appear important in their formation since hypocotyls without cotyledons will not produce any root hairs even when cultured on nutrient agar.

LAUBENGAYER, RICHARD A., Wabash College, Crawfordsville, Ind. *The vascular anatomy of the mature ear and tassel of Zea mays.*—There have been suggested several theories as to the nature and origin of the ear and tassel of corn. For the most part these theories have been based on external studies of various kinds of ears, along with cytological and breeding data. In order to study the vascular anatomy of the mature ear and tassel, plants bearing 8-rowed ears were selected. Several mature ears and tassels were studied from such plants. The vascular anatomy of the shank was found to be essentially that of the main stem. In the transition region from shank to ear, the bundle system reorganized into two distinct systems: a strong inner system which formed vascular supplies to the pistillate spikelets in a definite pattern, and an outer weaker system which enveloped the inner and supplied the glumes. In all specimens studied the vascular supply of the main axis and branches of the tassel, bearing the staminate spikelets, showed the same behavior as that of the vegetative stem. Comparison of the vascular systems of the mature ear and tassel showed no obvious relationship.

LEAKE, DOROTHY V., University of Oklahoma, Norman, Okla. *Studies of development and germination of aplanospores and zoospores of Basicladia crassa, Hoffman and Tilden.*—Specimens of *Basicladia crassa,* Hoffman and Tilden, found growing on the shells of *Chelydra serpentina,* were collected during the fall of 1938, the spring of 1939, and in 1946. The filaments did not grow under laboratory conditions unless the portions of horn to which the holdfasts were attached were removed from the carapace with them. In hanging

drop preparations *Basicladia crassa* was observed in process of producing aplanospores and zoospores. Germination of aplanospores and zoospores began within three or four days and was observed until the germlings had become two to several celled.

LIVINGSTON, L. G., AND I. W. BAILEY, Swarthmore College, Swarthmore, Pa., and Harvard University, Cambridge, Mass. *The demonstration of unaltered plasmodesmata in the cambium of Pinus strobus and in ray cells of Sequoia sempervirens.*—The numerous controversial points and divergences of opinion relative to the occurrence and true nature of plasmodesmata in plant tissue is largely due to the lack of a critical method for their study in an unaltered condition. The usual swelling techniques employed for their demonstration bring about profound alterations both in the cell wall proper and in the plasmodesmata present in the wall. Dehydration techniques bring about equal if not greater distortion of unlignified primary walls than properly controlled swelling. The only way to arrive at a satisfactory understanding of the true nature of plasmodesmata in the living plant is to study the cell walls in an unaltered state. In the studies here reported it has been possible to clearly demonstrate and study plasmodesmata in untreated sections cut from the living cambium of *Pinus strobus* in the winter condition and in the wood ray cells of *Sequoia sempervirens*. In these tissues, plasmodesmata are found to be universally present in all primary pit areas. The threads penetrating the walls from adjacent cells are intimately associated with one another if not actually continuous. The evidence available indicates that they are protoplasmic in nature. They are extremely minute structures, the diameter of the thread itself in these tissues not being much more than 0.2 μ. Preliminary observations on other plants would indicate that plasmodesmata are generally of the same nature and magnitude in the various tissues of higher plants. It is felt that reports of the presence of very large protoplasmic processes, several microns in diameter, interconnecting the cells of higher plants are due to the lack of critical observation of the wall in the region of the primary pit areas.

LIVINGSTON, L. G., AND CHARLES HEIMSCH, Swarthmore College, Swarthmore, Pa., and Amherst College, Amherst, Mass. *The use of leaf peel preparations in teaching leaf anatomy.*—Traditional leaf cross sections do not provide an adequate three dimensional understanding of leaf anatomy to the average student. While leaf peel preparations have been used extensively for epidermal study, their value for internal study has not been adequately appreciated. Only certain types of leaves will yield preparations suitable for study. Of more than forty species tried, *Aquilegia vulgaris* consistently yields the best preparations. The leaves are prepared by making a diagonal tear similar to the technique used in making peel preparations of the lower epidermis. The two opposing halves are placed in separate containers, and fixed with F.A.A. to which copper acetate has been added to preserve the green color of the chloroplasts. Temporary slides are made by mounting pairs of fragments oriented so that the exposed tissues of the torn surfaces are uppermost. With *Aquilegia,* the fragments mounted with the upper surface uppermost show extensive areas of sponge mesophyll cells, one cell in thickness, adhering to the inner surface of the lower epidermis as a clear two dimensional network. Veins are exposed to advantage, showing the relation of mesophyll cells to the bundle sheath, details of branching, spiral vessels, and various other features. Exposed palisade cells can be viewed from the end, and optically sectioned throughout their length. The intercellular space system is displayed to particular advantage. The fragments mounted with the lower surface uppermost show extensive areas of exposed sponge mesophyll which can be optically sectioned to demonstrate the three dimensional network nature of this mass of tissue. Veins and isolated patches of palisade tissue can also be viewed to advantage from below. With this type of preparation, the average beginning student can attain a sufficient understanding of leaf anatomy to enable him to construct accurate three dimensional models.

MARVIN, JAMES W., University of Vermont, Burlington, Vt. *Some factors influencing sap flow in Acer saccharum Marsh.*—In nature, day temperatures above 32°F. and night temperatures below 32°F. produce a pressure in the vessel sap of dormant maples which yields a flow through holes in the stem. Stevens has recently reported that excised stems yield a flow from the cut ends under these conditions. In the present investigations, small excised stems were exposed to controlled temperatures from 20°F. to 120°F. and the effect of temperature change on the vessel sap was studied. When frozen stems are warmed and held at a constant temperature, a flow occurs. There is considerable variation between stems in the amount and duration of the flow. After the cessation of flow at a constant temperature or after a temperature decrease, water is absorbed from the cut end. Other genera—*i.e., Fagus, Prunus, Tilia* and *Populus*—absorb water under the conditions which induce a flow of sap from maple stems.

MATZKE, EDWIN B., Columbia University, New York, N. Y. *The three-dimensional shape of epidermal cells of Aloe aristata.*—Epidermal cells of the upper surface of the leaf base of *Aloe aristata* were chosen for three-dimensional studies because they were relatively undifferentiated morphologically, and because they were underlain by cells of approximately similar dimensions. The tissue was cleared, stained, and examined *in toto*. The average number of faces for 200 cells was 10.885, the range being from 8 to 16. There were 83 cells with 11 faces. In general, larger cells had a greater number of faces. The faces varied from triangular to octagonal; 48.6 per cent were pentagonal, and 96.5 per cent were quadrilateral, pentagonal and hexagonal.

The proportion of hexagons in the outer faces was greater than in the lateral and basal faces. The 200 cells occurred in 39 combinations of faces. The commonest combination, 3 quadrilateral, 6 pentagonal, 2 hexagonal faces (3-6-2) was found in 34 cells. Other common combinations were 4-4-3, and 4-4-2. The epidermal cells were intermediate between peripheral bubbles in a uniform foam and peripheral compressed shot in average number of faces, in kinds of faces, in number of combinations of faces, and in number of tetrahedral angles. In specific combinations of faces peripheral bubbles in foam matched the epidermal cells more closely than did peripheral compressed shot. These results are interpreted as indicating that surface forces are of significance in epidermal cell shape determination, though less important than in foam.

MELHUS, I. E., G. SEMENIUK, AND J. R. WALLIN, Iowa State College-Guatemala Tropical Research Center, Antigua, Guatemala, C. A. *The influence of climate on distribution of corns in Guatemala.*—The variability in corn in Guatemala accounts for the general distribution of corn from sea level to 9500 feet altitude. Within this spread of altitude there is wide diversity in climate ranging from tropical to temperate at 7000 to 9500 feet. The corns in these climates fall into three groups based on leaf number and maturity: first, a short early corn with 9 to 14 leaves occurs in the highlands (3000 to 6000 feet); second, a mid-season corn with 15 to 21 leaves occurs in all climates, but is the chief corn in the lowlands and mountainous regions; and third, a large, long season corn with 22 to 35 leaves, requiring 5 to 7 months to mature, found only in the highlands. The early corns are less plentiful and limited in their climate range than the mid-season ones, which are the most common and generally distributed in all climates. The long season corn is limited to the highlands where a long uniform growing season prevails. The vigorous vegetative development of this group is distinctive and unknown in other groups. The mid-season corns in the tropical climate are not pigmented, while those growing in the mountainous climate show marked anthocyanin pigmentation of the leaf sheaths and stalks. Climate has exerted a selective influence on the distribution of corns of different leaf number and maturity in Guatemala.

MEYER, SAMUEL L., University of Tennessee, Knoxville, Tenn. *Spore germination and protonema development in Physcomitrium turbinatum (Michx.) Brid.*—In germination, spores of *Physcomitrium turbinatum* regularly produce a first filament that is slender and colorless, followed by a second filament which is heavier and chlorophyllose. The two filaments differ physiologically since the non-chlorophyllose thread is negatively phototropic while the chlorophyllose filament is positive in its light reactions. Though of the *Funaria*-type, spore germination in *Physcomitrium turbinatum* is more similar to that of the European species, *Physcomitrium pyriforme,* than to that of *Funaria hygrometrica.* Protonema development and gametophore formation in *Physcomitrium turbinatum* is of the usual type for members of the Bryales.

RICK, CHARLES M., University of California, Davis, Calif. *Studies of natural populations in the Nemophila Menziesii-atomaria complex.—Nemophila Menziesii* Hook. & Arn. (*sensu latu*), an annual hydrophyll native to the Pacific coast, includes a wide variety of biotypes, which have been treated in the past as separate species by some students, included in a smaller number of species, or even combined into a single species by others. Anderson's index of species hybridization was applied to eight morphological characters in mass collections and single specimens of material from Oregon and Northern California. Two main elements can thereby be distinguished: *atomaria,* which occupies the northern outer Coast Ranges, and *Menziesii,* which occupies most of the range of *atomaria* and also extends farther to the interior and to the south. Both are found in a pure state in at least part of the areas where they exist alone. In the region of overlapping ranges and also in part of the adjacent territory occupied by *Menziesii* alone, characters of one type infiltrate the other, the degree of infiltration of opposite characters into a type increasing approximately with depth of penetration of that type into the range of the opposite type. Even in populations in which both types intermingle, each retains its identity as shown by widely separated frequency distributions. All attempts to hybridize different collections of *atomaria* with those of *Menziesii* have failed, although most combinations within either type yield fertile hybrids. Of the eight characters, corolla color and receptacle shape show the closest agreement with the total indices and with compatibility relations.

ROLLINS, REED C., Stanford University, Calif. *The occurrence of sublethal dwarfed hybrids in Parthenium and their chimeric mutation to normalcy.*—Approximately one-half of the F_1 progenies of a reciprocal cross between *Parthenium argentatum* (36 chr. + several fragments) and *P. tomentosum,* var. *stramonium* (36 chr.) were exceedingly dwarfed and difficult to keep alive. The other half of the progeny consisted of healthy, vigorous hybrids. Among the dwarfed hybrids which lived, two plants gave rise to normal rapidly growing branches by chimeric mutation. There were $2n = 36$ chromosomes in both dwarfed and normal hybrids plus 0–5 fragments. The same range of fragment-number was found in both types. Thus the possibility of the presence or absence of a whole chromosome or of a fragment producing the dwarfing effect was eliminated. The evidence points to a simply inherited causal mechanism for the sublethal dwarfism found.

SANDERS, MARY, Genetics Experiment Station, Smith College, Northampton, Mass. *Growth of self and hybrid Datura embryos in culture media.*—Self and hybrid embryos involving four species of *Datura* were dissected out of the seeds at an early

stage of development (approximately 0.5 mm. or less) and grown in artificial media. The nutrient series represented three concentrations of sucrose, three concentrations of sodium metaphosphate (Calgon), and three concentrations of ferrous phosphate. Some of the embryos responded distinctly to the varying concentrations thereby indicating species or hybrid differences in physiological requirements. In general the growth of self embryos more or less followed normal development, and the growth of hybrid embryos was abnormal.

SATINA, S., A. F. BLAKESLEE, AND A. G. AVERY, Genetics Experiment Station, Smith College, Northampton, Mass. *Periclinal chimeras in Datura.*—Among some 1500 plants secured from seeds treated with colchicine, a random sample of 100 plants was selected for detailed study; 41 were diploid throughout and 59 were modified chromosomally. Of the latter 5 showed the same individual modification in all branches examined. The remaining 54 included various combinations of periclinal chimeras, mixochimeras, $2n$ and $4n$ branches; 30 were periclinal chimeras without any evidence of mixochimera tissue. Only 7 plants were periclinal chimeras throughout and from these only 2 plants showed the same type of chimera in all branches examined. Two plants showed 5 branches chromosomally different, 1 had 4 branches different, 15 had 3 different and 36 had 2 different.

SCOTT, F. M., AND K. BAKER, University of California, Los Angeles, Calif. *The anatomy of the Washington naval orange rind in relation to water-spot.*—Localized edema of the mature rind is the first symptom of water-spot injury, which may or may not be followed by rind-cracking during winter rains in California. Epidermal wax coating consists of scales. Wax is secreted through minute radial canals. The epidermis is structurally weak and liable to crack, due to thinly cutinised discrete new elements and circumstomatal accessory cells. The latter areas disintegrate readily in H_2SO_4. The albedo differentiates from meristematic tetrakaidecahedral cells and the mature open meshwork consists of 8-armed cells interconnected by plasmodesmata. The tendency of the epidermis to crack is evident in young fruits but incipient cracks are healed by concurrent cutinisation. Rind weakness becomes critical at maturity when epidermal extension fails to keep pace with internal expansion. Resultant cracks may heal in dry weather, but in continued rain remain open and admit fungal spores. Aqueous vital stains penetrate the mature rind readily and accumulate in the cell vacuoles of the accessory and certain other cells. Oil-spraying has no visible effect on the structure of the fruit. No significant anatomical differences are observed between the Washington naval and other varieties less susceptible to water-spot.

SINNOTT, EDMUND W., Yale University, New Haven, Conn. *Relative growth of pedicel and fruit in Cucurbita.*—In all types of Cucurbita studied, the pedicel attains its full growth considerably before the fruit does. The early part of pedicel growth is exponential but always at a rate less than that of the fruit. In all races, when the diameter of the fruit (corrected to spherical form) reaches about ten times the pedicel diameter, fruit growth ceases. Fruit growth rate is the same in large-fruited and small-fruited races but pedicel growth rate is markedly less rapid in the latter, so that the limiting ratio of fruit to pedicel is attained much earlier in small-fruited races than in large ones. This suggests that a factor in determining the duration of fruit growth, and thus fruit size, is the rate of pedicel growth, since where this is relatively slow, the amount of conducting tissue through which materials pass to the developing fruit is relatively small. Experimental reduction in stalk diameter, however, unless very severe, was without significant effect upon fruit size. Other correlative factors are discussed.

SMITH, BEN W., North Carolina State College, Raleigh, N. C. *Macrosporogenesis and embryogeny in Arachis hypogaea L. as related to seed failure.*—Only seven of 100 fertilized ovules survive to become well developed, mature seeds in a Jumbo running variety of peanut, *Arachis hypogaea* L. Sixty per cent of the potentially two seeded ovaries produce gynophores; 36 per cent of the gynophores bear well developed pods; 50 to 60 per cent of the pod segments contain normal seeds. Analysis of reproductive morphology accounts for part of the fruit and seed loss. Two, occasionally three, amphitropous ovules in virtually sessile ovaries contain solitary macrosporocytes. Meiosis is normal, $n = 20$. An 8-nucleate, 7-celled embryo sac, normal type, arises from the chalazal macrospore. Synergids and antipodals degenerate; mature embryo sacs contain the egg and a large endosperm mother cell. Double fertilization is completed 12 to 16 hours after anthesis. Ninety-five per cent of the naturally self-pollinated ovules are fertilized. The ovary base elongates, forming the gynophore which forces the ovules into the soil to a depth of 20 to 50 mm. Cell and nuclear division in embryo and endosperm proceed slowly; by 8 days after flowering embryos of 5 to 20 cells occur with endosperms of 8, 16, or 32 nuclei. Embryo and endosperm are more advanced in basal than apical ovules. Soil penetration occurs at 8 to 10 days. Rapid embryo and endosperm development begins with the onset of subterranean fruit enlargement, at 14 to 20 days. In 618 ovules representing 1 to 14 days of development, 74 (12 per cent) of the embryos were aborting. Sixty-two abortions occurred in apical ovules. Associated endosperms appeared normal. Prior endosperm failure does not condition early embryo abortion.

VAN FLEET, D. S., University of Missouri, Columbia, Mo. *Differentiation gradients in the cortex of vascular plants.*—Cortical cells are similar in general histological appearance, but it may be demonstrated that there are specialized patterns and gradients that indicate differentiation within

the cortex. By means of natural internal indicators and suitable oxidation-reduction indicators patterns in the cortex may be observed as redox and/or substance gradients. There are oxidation zones in the outer cortex, in the inner cortex, opposite the phloem and opposite the gaps in the endodermis. There are reduction zones in the middle cortex, opposite the xylem in roots, opposite root primordia and in some plants in the outer cortex. The oxidation of introduced indicators proceeds from the outside inward and from the stele outward at the same rate toward the middle of the cortex, a reduction zone. The pattern and gradient of oxidation of natural aglycones was found to correspond with the pattern of oxidation of other indicators. Acidic dyes are absorbed by the inner cortex. Basic dyes are absorbed by the outer cortex. The outer cortex in young roots reacts with nitroprusside, and the same cells reduce redox indicators. With polychrome methylene blue the middle cortical cells absorb azurin (red) molecules, and the outer and inner cortical cells absorb methylene blue (blue) molecules. The cortex opposite a root primordium is a reduction zone before the root emerges from the cortex. A gradient in uronic acid was found in the cortex of some roots.

VERDOORN, FRANS, Chronica Botanica, Waltham, Mass. *Publication problems and international cooperation in biology.*—One may say that there are seven groups of publications which serve to promote the progress of science: (1) publications reporting about the results of original research, (2) text, hand and reference books, (3) popular books and journals, (4) abstracting journals, (5) scientific news journals ("Fachblätter"), (6) directories and censuses of research, (7) bibliographies, encyclopedias, and indices. For each of these groups the principal current problems are outlined from the point of view of the biologist and with special reference to improvements which might be possible by closer international cooperation. It is emphasized that (1) biological books of limited interest, including lithoprint reprints of scarce old classical publications, can be published without too much loss if their distribution is well organized on an international basis, (2) there is an urgent need for an *international* newsmagazine in biology or (and) certain of its branches and, (3) international periodical censuses of current research in the biological sciences, (4) little can be accomplished along these lines without international non-political associations of plant and animal scientists with a membership open to individuals of all countries.

WARMKE, H. E., AND GERMAINE LE CLERC WARMKE, Institute of Tropical Agriculture, University of Puerto Rico, Mayagüez, P. R. *The role of auxin in the differentiation of roots and leaves in Taraxacum.*—From a genetic point of view an understanding of the mechanisms which enable two cells of the same plant to differentiate into such diverse structures as roots and leaves is of fundamental importance. Recent studies in this laboratory indicate that auxins, which are already known to regulate many vital plant processes, also play an important role in root vs. leaf differentiation. If the fleshy taproots of *Taraxacum* or *Chicorium* are cut into short segments, these will regenerate into complete plants—leaves differentiate from the proximal cut surface, and callus and sometimes roots differentiate from the distal cut surface. This natural regeneration can be profoundly altered, so that roots differentiate from both distal and proximal ends, by the application of indolebutyric acid in concentrations of 100–400 p.p.m. Conversely, leaves can be caused to differentiate from both ends of the segments if the auxin content is lowered below its normal level, by prolonged washing or by treatment with chemicals such as ethylene chlorohydrin that are known to reduce auxin content. The direction of differentiation of the callus tissue which forms on the cut surfaces of this material is thus under experimental control; either roots or leaves can be caused to develop, depending on whether the auxin content is increased or decreased.

WEATHERWAX, PAUL, Indiana University, Bloomington, Ind. *Primitive characteristics in a Peruvian variety of maize.*—A variety of corn found in the Peruvian Andes has a number of characteristics which are regarded as primitive for the species. Among these are: axillary ear branches, indeterminate pistillate inflorescences, mixed inflorescences, vegetative branches of the main stem, and two-flowered pistillate spikelets.

WHITFORD, L. A., North Carolina State College, Raleigh, N. C. *Structure and composition of a recently described fresh-water alga; Phaeosphaera perforata (Chrysophyceae).*—Microscopic globular colonies consisting of a few cells attached to rocks in streams expand into ovoid and soon cylindrical colonies perforated by circular openings of many sizes. The body of the colony is composed of a clear pectic material in which nearly globular cells are irregularly distributed. The pectin is apparently secreted as filaments and stains show it to form a denser layer around each cell. The cells are without walls and filled with clear cytoplasm frequently having one or two contractile vacuoles. There is a single nucleus about one-third cell diameter with a large caryosome. The chromatophore is parietal, golden-brown and covers about one-fourth to one-third the cell surface. There are at least four pigments in the chromatophore. A golden-yellow water-soluble pigment (chrysochrome?) has a spectrum absorption maximum at 440 mμ with no minimum in the visible spectrum. Apparently there are two chlorophylls and in addition a carotinoid pigment. Food reserves are clear globules of various sizes outside the chromatophore. Tests indicate these are oil or probably sometimes leucosin. Smooth spherical cysts (stratospores) somewhat larger than vegetative cells and with a silica wall and pore are formed from vegetative cells. The cytoplasm in young cysts is vacuolated and reticulate. Oil droplets are sometimes present. Two nu-

clei occasionally are present and in one case these appeared to be fusing. Naked pyriform to reniform zoospores are also formed from vegetative cells. There is one laterally attached flagellum about twice the cell length. The zoospores have a small chromatophore but no demonstrable food reserves. The largest intact colony collected measured 5 by 15 cm. Old colonies become torn and detached and may grow for a time in quiet pools. These form highly reticulated irregular sheets.

WITKUS, E. R., Fordham University, New York, N. Y. *Additional evidence on the role of polyploidy in plant development.*—A new case is here reported in which the normal developmental pattern of a diploid plant involves the formation of some tetraploid cells in certain definite regions. The largest cells in the periblem of seedling roots of *Mimosa pudica* were found to be tetraploid and to undergo mitosis as tetraploids. The tetraploid condition arose during the preceding resting stage by a process of double reduplication of chromosomes within the resting nucleus. Evidence for this method of origin was found in the closely paired condition of the chromosomes throughout prophase and at metaphase in tetraploid divisions. The process in *Mimosa* is similar to but not identical with the phenomenon termed "polysomaty" found in *Spinacia.*

WOODS, M. W., AND H. G. DUBUY, University of Maryland, College Park, Md., and Berwyn, Md. *Oxidizing enzymes in variegated leaves and in isolated normal and mutant plastids.*—Colorless mutated plastids in variegated *Hydrangea* sp. leaves are associated with a pronounced hypoplasia. In *Lonicera japonica* the light green to yellow mutant plastids do not markedly interfere with cell growth. Oxygen uptake by both chlorotic and green areas in mature leaves of both plant species was not inhibited by 10^{-5} or 10^{-3} molar HCN. On the basis of equal weights of fresh mature leaf tissues, variegated areas usually displayed about the same or lower (17 to 29 per cent less) rates of oxygen uptake than corresponding normal tissues of both species. Whole leaf extracts likewise gave much lower catechol oxidase (59 to 85 per cent less) and catalase (34 to 67 per cent less) activities in variegated tissues. On the other hand peroxidase activity behaved differently. In *Hydrangea* the chlorotic tissues were from 30 to 50 per cent more active than corresponding green areas. In *Lonicera* chlorotic areas displayed from 30 to 40 per cent less activity than corresponding normal tissues. Centrifugally isolated mutant and normal plastids of both species displayed with one exception relative activities of the three enzymes which were similar to those of the whole leaf extracts. In *Hydrangea* no catechol oxidase activity could be detected in the isolated colorless plastids whereas the whole leaf extracts of variegated tissues always had a low activity. The enzyme activities appear to be closely associated with the plastids as they are not removed by repeated washing and centrifugation. Activity is destroyed by heating to 100°C. for 1 minute and is strongly inhibited by 10^{-3} M. HCN. Observations in variegated sweet potato (var. Nancy Hall) show that peroxidase activity can be hypernormal without pronounced change in catechol oxidase activity.

YOCUM, CONRAD S., University of Maryland, College Park, Md. *The relation between respiration and chlorophyll formation.*—Chlorophyll formation in angiosperms is affected by many factors which affect respiration. In view of this apparent relation the rates of these two processes were compared in excised leaves of dark grown bean seedlings. Carbon monoxide-oxygen mixtures (95%: 5%) reduced the O_2-uptake in the dark to about 60 per cent of that in 95% nitrogen–5% oxygen mixtures. The rate of chlorophyll formation in red light in the same carbon monoxide mixture was reduced approximately 50 per cent during 24 hour periods. Strong blue light completely reversed monoxide inhibition of both processes. 0.001 M cyanide inhibited O_2-uptake approximately 66 per cent, whereas it had no effect on the rate of chlorophyll formation during the first hour of illumination. At the same time there was a decrease in protochlorophyll concentration as measured by the percentage absorption at 6300 Å in ether extracts. After this induction period 0.001 M cyanide completely blocked chlorophyll formation. Neither cyanide nor monoxide changed the ratio of chlorophylls "a" and "b". These experiments show that the photochemical conversion of protochlorophyll to chlorophyll is insensitive to cyanide, but that protochlorophyll *synthesis* cannot proceed in the presence of 0.001 M cyanide. Since protochlorophyll formation is dependent on the functioning of a respiration system which shows a reversible cyanide and a light reversible CO inhibition it is suggested that cytochrome oxidase is essential for protochlorophyll synthesis.

MICROBIOLOGICAL SECTION

(Abstracts of this Section are arranged alphabetically according to author)

AJELLO, LIBERO, Columbia University, New York, N. Y. *Studies on the nutritional requirements of Polychytrium aggregatum Ajello, a chitinoclastic chytrid.*—With the isolation of *Polychytrium aggregatum* in pure culture on a peptone, dextrose, yeast extract medium, a study was made of the nutritional requirements of this fungus. *Polychytrium aggregatum* is able to grow in a vitamin-free medium, indicating an ability to synthesize all the vitamins required for its growth. Both organic and inorganic compounds can be utilized as nitrogen sources. Chitin is utilized as a nitrogen source but the carbon present in the chitin molecule apparently is not available. The optimum tempera-

ture and hydrogen ion requirements for this organism were determined. Degrees of growth with the various test compounds were determined by dry weight yields.

BLACK, L. M., The Brooklyn Botanic Garden, Brooklyn, N. Y. *Variation in host susceptibility to wound-tumor virus.*—Wound-tumor disease, which shows homologies with certain animal tumor diseases, is caused by a virus which is spread by specific insect vectors; it is not transmissible from plant to plant by mechanical methods. Because wounds start tumors of potentially unlimited growth in plants systemically invaded by the virus, it has been named wound-tumor disease. The present report bears on the influence of heredity of the host plant on tumor response. In a preliminary experiment six genetically different lines of sweet clover were established by making cuttings of plants showing a considerable range in morphological characters; at least seven young plants of each clone were successfully inoculated with wound-tumor virus by means of the insect vector, *Agallia constricta.* The root-tumor responses of the different clones were extremely variable while the infections within the plants of a single clone were remarkably uniform. In another experiment two plants of each of thirty-six inbred lines were inoculated with the virus. The root-tumor response of the two plants in each line was very similar. In the different genetic lines, however, the number and size of tumors varied greatly. Previous work has demonstrated that two factors, virus and wounds, play an important part in tumor production in this disease. The present results indicate the importance of a third factor, namely, the heredity of the host.

BONNER, JOHN T., Harvard University, Cambridge, Mass. *The role of diffusion in the aggregation stage of the slime mold Dictyostelium discoideum.*—*D. discoideum* is a member of that group of slime molds (Acrasiales) which forms its fruiting structure from a compacted mass of uninucleate myxamoebae known as a pseudoplasmodium. The pseudoplasmodium arises by the aggregation of many myxamoebae which were previously completely independent and separate from one another. In this so-called *aggregation* stage one can see radial streams of elongate amoebae move towards a central point by means of pseudopodial locomotion. Attempts to discover the immediate cause of this centripetal streaming of myxamoebae revealed that it was not an agglutination process; that a spreading surface film phenomenon was not responsible; that an electrical or magnetic force was improbable; that any form of directing ray was not involved; and that no type of predetermined structural matrix existed that could guide the myxamoebae to the center. There is, however, clear evidence that an unknown substance diffuses from a central mass of myxamoebae through the liquid medium and that the incoming myxamoebae orient themselves in the diffusion field, moving towards the point of highest concentration. This can be shown when aggregation takes place under a gentle stream of water for the current causes an asymmetry in the usually radial pattern of incoming myxamoebae that can only be interpreted as the warping of the diffusion field of a substance to which the myxamoebae are sensitive.

BUELL, C. B., AND W. H. WESTON, Biological Laboratories, Harvard University, Cambridge, Mass. *Application of the mineral oil conservation method to maintaining collections of fungous cultures.*—In connection with maintaining approximately 4500 cultures of fungi concerned in the deterioration of material in the tropics the method of long-term conservation of cultures by covering the agar slant with mineral oil was found valuable. This method, previously developed by workers in other fields, has been applied in the past to cultures of filamentous fungi in only a few cases. For conserving the viability of a total of about 1800 cultures of this collection the method proved to have certain notable advantages. Among these are: its elimination of frequent, repeated transferring, its simplicity which makes it useful for small as well as large collections, its applicability to fungi which will not tolerate lyophilization, and its effectiveness for eliminating mites. The advantages and disadvantages of this method of prolonging the life of fungous cultures will be compared with those of the lyophil technique.

BURKHOLDER, PAUL R., AND NORMAN H. GILES, JR., Osborn Botanical Laboratory, Yale University, New Haven, Conn. *Production of biochemical mutants in Bacillus subtilis by means of ultra-violet radiation.*—Suspensions of vegetative cells or spores were irradiated with a quartz mercury arc so as to kill about 99.9 per cent of the cells. Spores were then plated out prior to germination, while vegetative cells were incubated in shaking culture for 3 hours previous to plating. Approximately 2 per cent of 2000 surviving cultures tested were found to be deficient for growth factors. Among those which have been identified are mutants singly deficient for arginine, cystine, histidine, leucine, methionine, threonine, tryptophane, riboflavin, guanine and uracil. Approximately 1000 unirradiated controls failed to yield any detectable biochemical mutants.

EDWARDS, G. A., C. BUELL, AND W. H. WESTON, Biological Laboratories, Harvard University, Cambridge, Mass. *The influence of mineral oil on the oxygen consumption of Sordaria fimicola (Ascomycetes).*—In connection with the application of the mineral oil method to conserving cultures of fungi, as reported by Buell and Weston, it seemed desirable to follow over a period of time the metabolic rate of a culture so treated by means of measuring the oxygen consumption. The oxygen consumption of a culture of *Sordaria fimicola* was determined by means of a volumetric micro-respirometer. The QO_2 of a normal culture was found to be constant over a period of days after optimal growth was reached. Under oil the oxygen con-

sumption dropped rapidly within the first few hours, reaching a rather steady state. Thereafter the QO_2 decreased only slightly over a long period of time. During the course of the experiment the influence on the QO_2 of various factors, such as temperature and type of growth, was noted. The results indicate that it is possible, quickly and easily, to determine the metabolic state of a culture under oil in the manner discussed, thus obtaining an indication of the condition of the culture and the length of time the culture might be expected to remain viable.

GARDNER, ELIZABETH B., Radcliffe College, Cambridge, Mass. *pH changes in medium associated with growth habit of Aspergillus giganteus.*—Sporulation and elongation of conidiophores of *Aspergillus giganteus* Wehmer grown on a potato maltose medium is associated with interesting pH changes in the medium. The pH of the medium after autoclaving ranges around pH 5.7. During growth of substratal mycelium, the pH drops toward the acid end of the scale, reaching the vicinity of 4.8. Production of small spore heads close to the medium and/or elongation of tall conidiophores is accompanied by a trend toward the alkaline, reaching as high as 8.6 by maturity. Fifty per cent concentration of sodium phosphate-citric acid buffer solution is insufficient to hold the medium at the original pH level. This concentration of buffer does not appreciably affect normal growth habit. Pretreatment of the medium by allowing young cultures to start in the medium, removing these, autoclaving, and re-inoculating, tends to increase elongation rate of tall conidiophores. Submergence of young cultures with nutrient broth increases elongation rate after the submerging fluid is poured off. Observations suggest production by substratal mycelium of acids which are used by the aerial hyphae in spore production and elongation of tall conidiophores.

GOYAN, FRANK M., JEAN DUFRENOY, LOUIS A. STRAIT, AND ROBERTSON PRATT, University of California College of Pharmacy, San Francisco, Calif. *A three hour "physical development" cup-plate assay for penicillin.*—The paper describes a practical procedure for assaying penicillin in three hours. The procedure retains the advantages of the cup-plate method but eliminates its serious disadvantage, *i.e.*, the 18-hour period of incubation. Additional advantages of the present 3-hour method are linearity of the standard calibration curve and extension of the useful range of the curve to nearly twice that of the conventional method. The calibration curve for the 3-hour method covers the range from 1 to 8 units/ml. and may be extended from 0.5 to 10 units/ml. with but slight error. The present method employs the principles of photographic development. The seeded layer of the test plates is made analogous to a photographic emulsion by impregnation with silver. Since cells of *Staph. aureus* and of *B. subtilis* exposed to penicillin differ from normal cells in their affinity for and subsequent chemical reduction of silver, exposure to light produces different latent images in the areas of inhibition and non-inhibition. Subsequent physical development reveals clearly the boundary between the two areas.

GRAY, W. D., AND G. W. MARTIN, Iowa State College, Ames, Iowa, and University of Iowa, Iowa City, Iowa. *The growth of fungi on asphalt-treated paper.*—Asphalt-coated paper is extensively used to protect the contents of boxes containing materials subject to injury by molds. It is sometimes assumed that such papers are highly resistant to mold growth. Experimental evidence is presented showing that such is not the case and suggestions are made for use of inhibitors to improve the value of asphalt-treated paper for the purpose mentioned.

HOLLAENDER, ALEXANDER, C. P. SWANSON, AND IRENE POSNER, National Institute of Health, Bethesda 14, Md. *The sun as a source of mutation producing radiation.*—The ultraviolet radiation emitted by the sun and reaching the surface of the earth contains sufficient intensities at wavelengths shorter than 3100 Å to make it a possible source of mutation producing energy. During the summer months of 1946 in the Washington area it was possible to demonstrate the efficacy of sunlight in producing mutations in *Aspergillus terreus* and *Trichophyton mentographytes*. The rate of mutation production is rather low, not exceeding 4 per cent of the surviving spores and averaged approximately 2 per cent. The survival ratio and rate of mutation were roughly correlated with the ultraviolet intensity measurements made simultaneously at the National Bureau of Standards by Dr. W. W. Coblentz.

KOFFLER, H., F. H. JOHNSON, AND P. W. WILSON, Department of Agricultural Bacteriology, University of Wisconsin, Madison, and Department of Biology, Princeton University, Princeton, N. J. *Respiration of Rhizobium in relation to temperature and urethane.*—The success with which the theory of absolute reaction rates has been recently applied to the quantitative interpretation of bioluminescence (Eyring and Magee, 1942; Johnson, Eyring, Steblay, Chaplin, Huber, and Gherardi, 1945) encouraged this attempt to analyze respiratory processes in a similar manner. Such an analysis supports the views that (1) at temperatures below the optimum or slightly above it, the respiratory rate of "resting" cells of *Rhizobium trifolii* is affected by an equilibrium between catalytically active (native) and inactive (denatured) forms of their respiratory enzymes; (2) ethyl carbamate (urethane) inhibits respiration by combining, reversibly at lower temperatures and irreversibly at higher temperatures with the denatured form of these enzymes, thus causing a shift in the normal denaturation equilibrium and a decrease in the concentration of active enzymes.

KUNKEL, L. O., The Rockefeller Institute for Medical Research, Princeton, N. J. *Incubation pe-*

riod of corn stunt virus in the leafhopper Baldulus maidis (De L. and W.).—Baldulus maidis readily transmits corn stunt virus but it is never able to do this immediately after first feeding on a diseased plant. An interval varying from about 16 to more than 30 days must elapse between the time when the leafhopper first feeds on diseased tissues and the time when it is first able to transmit. This waiting interval, which is known as the incubation period of the virus in the insect, is shortest during summer months when temperatures are high. Under winter conditions in greenhouses held at about 75°F. the waiting period exceeds one month. The incubation period of corn stunt virus is one of the longest that has been reported.

LILLY, VIRGIL GREENE, AND H. L. BARNETT, Dept. of Plant Pathology and Bacteriology, West Virginia University, Morgantown, W. Va. *The inheritance of partial thiamin deficiency in Lenzites trabea (Pers.) Fries.*—Single spore isolates from the same fruit body of *Lenzites trabea* varied as much as four fold in amount of growth when cultured in the absence of thiamin. The derived dicaryon strains also showed similar variation in growth in the absence of thiamin. In all cultures tested growth was improved by the addition of thiamin to the medium. The F_1 haplonts of a few crosses were studied in detail. In general a few of the F_1 haplonts resembled one or the other haplont parent, while many were intermediate. Evidence is presented which strongly indicates that the ability to make good growth in the absence of added thiamin is inherited, but it does not follow the pattern of simple Mendelian inheritance.

NICKERSON, WALTER J., Wheaton College, Norton, Mass. *Oxidative metabolism of Candida albicans.*—Oxygen consumption as determined by the Warburg technique showed a decline in the endogenous rate (absence of added substrate) with increasing age of cells. The endogenous rate decreased with starvation; the decrease was most pronounced with suspensions of young cells. Suspensions of cells prepared from 18 hour cultures grown with aeration in liquid media oxidized added glucose readily; this exogenous rate declined with increasing age of the culture and with starvation. The endogenous R.Q. was 1.0 for all age cultures and all starvation times; the exogenous R.Q. was consistently much less than 1.0 with glucose in all experiments, except in the presence of added riboflavin. M/600 to M/1000 dinitrophenol and 2-amino, 4-nitrophenol increased endogenous rates up to threefold in suspensions starved as long as 8 days. These compounds did not increase the exogenous rate of oxygen consumption but did prevent oxidative assimilation in young cells. Riboflavin (0.25 μg/ml) markedly inhibited the endogenous rate and decreased the extent to which glucose was oxidized (but not the rate of oxidation)—the reverse of the effects of dinitrophenol. At this concentration riboflavin completely overcame the effects of added dinitrophenol. It is well known that some of the non-pathogenic species of *Candida* can produce very high concentrations of riboflavin, excreting it into the culture medium. *C. albicans* appears unable to do this but is known to produce a green pigment, apparently other than riboflavin, under some conditions. These relationships are being investigated in light of the above findings.

PERLMAN, D., Research Laboratories, Merck and Co., Inc., Rahway, N. J. *The nutrition of Memnoniella echinata.*—The organism was found to utilize some twenty sugars and sugar derivatives as sole sources of carbon for growth. The main product of glucose fermentation was carbon dioxide; traces of acetic acid were also formed. The nitrogen sources found to give good growth included: ammonia-N; nitrate-N; amino-N; imidazole-N; pyrimidine-N; purine-N; and pyrrolidine-N. Growth was increased when traces of iron, zinc, manganese, and chromium were added to the synthetic medium. Marsh has shown that biotin is the only growth factor required for maximum growth on synthetic media. In these experiments the requirement was 3 gammas per liter. Synthetic *dl*-desthiobiotin was inactive as a biotin substitute, while *dl*-O-heterobiotin had 10 per cent the growth promoting activity of *d*-biotin (on a weight basis). Mixtures of *d*-desthiobiotin and *d*-biotin had 130 per cent the growth promoting activity of an equal amount of biotin; *d*-O-heterobiotin—*d*-biotin mixtures had 60 per cent biotin activity on the same basis. The molar inhibition ratio of γ (3,4-ureylenecyclohexane) butyric acid toward biotin and O-heterobiotin was approximately 10,000. When aspartic acid was added to the medium, the ratio toward *d*-biotin was 100,000, and the biotin requirement for maximum growth was decreased to 0.1 gammas per liter.

REESE, ELWYN T., Botany Department, Pennsylvania State College, State College, Pa. *Aerobic decomposition of cellulose by micro-organisms at temperatures above 40°C.*—Four cellulose decomposing organisms, isolated from the aerobic warmer regions of a composting horse manure pile, were studied by the shaker-flask technique. The organisms were *Humicola grisea, Aspergillus fumigatus, Actinomyces thermophilus,* and *Coprinus* sp. The first two were most active, at 45°C. causing a 40–50 per cent loss in cellulose in 4 days. This rate is no greater than that previously obtained under identical conditions for similar organisms at 30°C. Small amounts of yeast extract stimulated all four organisms. Except for *A. fumigatus,* glutamic acid was able to replace the yeast extract on an equal weight basis. *A. fumigatus* was selected for enzyme studies. The cellulase activity of cell-free filtrates was found to be about equal to that of snail gut as reported by Karrer.

REESE, ELWYN T., Tropical Deterioration Laboratory, Philadelphia Quartermaster Depot. *Studies on the effect of aeration and nutrition on cellulose decomposition by certain bacteria.*—The shaker-flask technique which has had such increasing

application recently in the field of antibiotics has been applied to a study of cellulose decomposition. The filtering difficulty, common to residues resulting from microbiological action, has been avoided by using small amounts of filter paper (100 mg.), and by treating the residue with hot dilute KOH. When tested by this method, two species of bacteria (*S. myxococcoides, Cellulomonas sp.*), representative of those isolated from rotting textiles, brought about losses in weight of cellulose of at least 60 per cent in 4 days. Experiments on the effect of several factors on the rate of decomposition showed the method to be capable of detecting slight differences in environmental conditions. Investigations of aerobic cellulose decomposition, formerly requiring long periods of time, can now be greatly accelerated. Fungus degradation can be studied in a similar fashion.

SWANSON, C. P., AND ALEXANDER HOLLAENDER, National Institute of Health, Bethesda 14, Md. *Modification of the ultraviolet mutation rate by pretreatment with near infrared.*—Fungous spores irradiated with monochromatic ultraviolet shorter than 3100 Å show an increased mutation rate with increasing energy up to a certain maximum. The mutation rate declines if the energy is increased further. Near infrared radiation (6000 to 18000 Å) given alone does not produce mutations. However, if the infrared precedes the ultraviolet irradiation the mutation rate is lower for low energy values and higher at very high energies, *i.e.*, the maximum of the mutation curve is shifted towards higher energy values, than if ultraviolet is given alone.

THOMPSON, R. H., Department of Research and Education, Solomons Island, Md. *New records and species of immobile Dinophyceae.*—Six species are presented in this report representing three genera. In the first of these *Phytodinium simplex* Klebs has been known only from the original description. *Phytodinium ovum* sp. nov. was found first in Maryland and more recently in Massachusetts. The remaining two genera are new to science and each contains two species: *Acanthodinium bicorne* sp. nov., found in Maryland and Massachusetts, and *Acanthodinium octocorne* sp. nov., found in Maryland; and, *Pulvidinium quadratun* sp. nov. and *Pulvidinium tumidulum* sp. nov., both from Maryland.

WESTON, W. H., Biological Laboratories, Harvard University, Cambridge, Mass. *Some microbiological aspects of the fungi concerned in tropical deterioration of materiel.*—The collection of nearly 4500 cultures isolated from materials such as tentage in use or undergoing test exposure in the tropics and assembled, identified and maintained at Harvard during the past two years apparently constitutes a fair representation of the fungi concerned in such deterioration. Preliminary study of this collection brings out points of general microbiological interest, some of which will be presented in this paper. The proportionate representation of various classes, orders, and other taxonomic groups; the frequency of representation of various genera and species; and the part played by these fungi in the complicated picture of deterioration, will be mentioned. Preliminary evidence as to the geographic distribution of these fungi will be drawn from comparisons with similar collections from different localities. The occurrence in this collection of several new genera and species of mycologic interest will be noted. It will be noted also that this is not a specialized flora distinctive or restricted with respect to locality, substrata or activities, but a conglomerate, heterogeneous assemblage comprising components from many other floras and including fungi better known in other capacities, fungi of the soil, of decaying plant remains, of dung; fungi pathogenic to plants and to animals; fungi that are the excitants of inhalant allergies or the sources of antibiotic substances of therapeutic value.

WHITE, W. LAWRENCE, AND RICHARD T. DARBY, Philadelphia Quartermaster Depot, Philadelphia 45, Pa. *Cellulolytic activity of molds isolated from military fabrics exposed in the tropics.*—One of the first phases of the more purely mycological aspects of a broad program directed toward an understanding of the underlying nature and causes of the rapid breakdown of cotton military fabrics in the tropics consisted of the isolation of organisms present and a laboratory survey of their activity followed with more intensive study of a selected few. The present note deals with the comparative cellulolytic activity of species and genera based on the assay of approximately 420 cultures. The basic method used was that of Greathouse, Klemme, and Barker, into which was introduced several minor variations. The decline in tensile strength of strips of cotton fabrics on which the organism is incubated is taken as the measure of cellulolytic activity. All members of the Mucorales tested gave results that definitely were negative. Of approximately 100 cultures of *Penicillium* representing 27 named species and many unidentified ones, all gave negative results except those determined as non-ascosporic strains of the *P. luteum* series. Of 19 species of *Aspergillus* represented by 118 cultures, only the following species were found to be cellulolytic: *A. Fischeri, A. flavipes, A. fumigatus, A. terreus, A. ustus,* and 1 culture out of 30 in the black group. In *Pestalotia* 8 cultures were positive as contrasted with 1 negative. All cultures in the following genera were cellulolytic: *Alternaria, Brachysporium, Chaetomium, Gliocladium, Gliomastix, Humicola, Memnoniella, Myrothecium, Stachybotrys, Thielavia,* and *Trichoderma.* The percentage of cellulose-destroyers in *Fusarium* runs very high. Many cultures tested fall into relatively unknown genera not mentioned here, and some are still unidentified even to genus. These pure culture results must not of themselves be interpreted as an index to the economic significance of a particular species or group of species in the deterioration of fabrics under the complicated interacting factors of field exposure and where activity

other than cellulytic may enter into the picture. There is reason to believe that numerous species that may be commonly isolated from fabrics are of little significance and that a high percentage of the damage will eventually be demonstrated to be due to the activity of a relatively few species.

YOUNG, GENEVIEVE, Boston University, Boston, Mass. *The relation between pigment production and antibiotic activity in cultures of Pseudomonas aeruginosa.*—*Pseudomanos aeruginosa* was found to produce pyocyanin and alpha-oxyphenazine when grown in potato-glycerol broth. In 1 per cent glycerol broth, or 0.5–1 per cent dextrose broth, a fluorescent pigment was produced in addition to these two. Veal infusion broth yielded only small amounts of pigments, and dextrose broths containing more than 1 per cent sugar showed no pigment formation. Antibiotic activity was determined for heated whole cultures, pyocyanin, alpha-oxyphenazine, other ether-soluble substances, and culture residues after chloroform and ether extraction. *Staphylococcus aureus, Escherichia coli,* and *Mycobacterium smegmatis* were used as test organisms. All three were inhibited by pyocyanin. Alpha-oxyphenazine, ether extracts of cells, and fluorescent culture residues inhibited *Staph. aureus* and *M. smegmatis.* Non-fluorescent residues were ineffective. Cultures producing no pigment showed no antibiotic activity, even on prolonged incubation, although growth of *Ps. aeruginosa* was heavy.

PALEOBOTANICAL SECTION

(Abstracts of this Section are arranged alphabetically according to author)

ANDREWS, HENRY N., The Missouri Botanical Garden, St. Louis, Mo. *Some fossil plant localities in Idaho and Wyoming.*—During May and June of last summer we returned to Idaho partly to continue collecting activities in the Cretaceous *Tempskya* fields of southeastern Idaho and partly to investigate certain other fossil plant deposits. A few days were spent in the Gallatin corner of Yellowstone Park studying the fossil forests in that region. As many as sixteen successive forests were counted and petrified trunks located up to 14 feet in diameter. In the little-studied late Tertiary beds south of Bruneau, in southwestern Idaho, a few petrified conifer cones were collected and a fine specimen of a *Fomes* (or *Polyporus?*) sporophore. A collection of leaf impressions were made near Rockville, Oregon, and a considerable number of *Tempskya* specimens were obtained in the vicinity of Wayan and Ammon, Idaho.

BANKS, H. P., Acadia University, Wolfville, Nova Scotia. *Protolepidodendron and Psilophyton from the middle Devonian of New York.*—A specimen of *Protolepidodendron* in the Goldring collection at the New York State Museum, Albany, appears to be the first collection of this genus in the U. S. A. that can be identified on the basis of clear-cut *external characteristics.* The material comes from the Hamilton (Moscow) beds at Gilboa, New York. A little-known species of *Psilophyton, P. grandis* Penhallow 1893, is reported from the Bellvale sandstones, marine Middle Devonian deposits of Marcellus or in part Skaneateles age, near Greenwood Lake, Orange County, New York. This species is compared with other valid species of the genus and it is pointed out that there are relatively few specimens that really fit the generic description in spite of the many specimens that have been labeled *Psilophyton.*

RADFORTH, NORMAN W., McMaster University, Hamilton, Canada. *Analyses of fossil plant compressions from Holland.*—Structural features revealed on the application of the transfer method are discussed as possible criteria in taxonomic designation.

SCHOPF, JAMES M., U. S. Bureau of Mines, Pittsburgh, Pa. *Botanical aspects of coal petrology; coal from the Coos Bay field in southwestern Oregon.*—The subbituminous coal of the Coos Bay field is classed as normally banded but it has a more uniformly bright luster than most varieties of banded coal. Some brilliant bands are obviously woody in derivation, but other zones of nearly equal luster are not as readily classified. Microscopic study shows that much of the brighter coal is derived from xylem in extreme stages of degradation; a few types of preservation are characteristic. One is typified by numerous inclusions interpreted as humic casts or "fillings" of angiospermic tracheae; other fragments have been infiltrated by yellow translucent waxy material; much of the woody tissue is simply compressed with no intercalation of extraneous substance. The most evident microfossils are the remains of fungi; sclerotia occur in particular abundance. These generally are uncompressed and yellow waxy material commonly fills the cellular interior. Fusain is virtually absent from much of the Coos Bay coal. A few inferences may be drawn concerning environmental conditions during the peat stage of coal formation.

PHYSIOLOGICAL SECTION

(Abstracts of this Section are arranged alphabetically according to author)

BIALE, J. B., University of California, Los Angeles, Calif. *Critical oxygen concentrations for the carbon dioxide evolution by citrus fruits.*—Uniform samples of lemons and oranges were subjected to modified atmospheres varying in oxygen content from 0 to 100 per cent at a constant temperature of 15°C. In some tests Q O_2 was measured in addition to Q CO_2. The standardized method of the triple response of pea seedlings was used to determine the production of active volatiles in relation to respiration. Under the limiting condition of the complete absence of oxygen it was found that the rate of CO_2 production was high for the initial 3 to 4 weeks. During this period the minimum Q CO_2 took place at oxygen tensions varying from 0.5 to 5 per cent. Above this range the Q CO_2 and Q O_2 increased with increasing oxygen concentrations. There appeared to be a range rather than a single value for the critical oxygen tension. The occurrence of the "climacteric," that is of the sharp rise in respiration characteristic of the senescence stage, was particularly noticeable under oxygen conditions higher than in air. It could be observed also at sub-atmospheric oxygen tensions, especially when the storage period was prolonged. This typical rise in Q CO_2 and Q O_2 was associated with the production of an active emanation as shown by the pea test. The changes in oxygen tension brought about differential rates of chlorophyll destruction in green fruit. Lemons subjected to air became fully yellow in 4 to 6 weeks, while those under 5 per cent O_2 or less had a considerable amount of green pigment in the peel even after 6 months. The storage life was also markedly prolonged by sub-atmospheric oxygen conditions.

CURTIS, O. F., AND D. G. CLARK, Cornell University, Ithaca, N. Y. *Effect of SO_2 in preventing heating, molding, and loss of dry matter in the curing of hay.*—In the barn-drying of hay in which the hay is placed in the mow over flues through which air is blown to finish the drying difficulty is often encountered especially during humid periods, that there is considerable molding of the hay and therefore loss in quality and food value. We have found on a small experimental scale that passing a single charge of SO_2 through the hay by introducing it through the blower system may be entirely effective in checking the growth of molds and in preventing the heating of the hay. With very little visible molding there may be 10 to 20 per cent loss of total dry weight as a result of natural heating over a period of 24 to 48 hours. With severe molding as much as 30 to 40 per cent of the original dry matter may be lost. These losses may be almost completely prevented by the gas treatment which appears also to prevent losses of carotene as well as carbohydrate.

CURTIS, O. F., JR., Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. D. A., Indio, Calif. *Diurnal translocation of sugars into date fruits.*—After a date fruit has reached its maximum size in terms of volume and fresh weight, the major portion of the dry weight is added in a period during which sugar accumulates rapidly until it comprises perhaps three-fourths of the dry weight of the ripe fruit. As an introduction to a study of the factors controlling the influx of sugars at this important stage, an attempt was made to ascertain the time of day during which translocation into the fruit occurred. The results of a preliminary survey, covering a total of eight diurnal cycles, indicate that the dry weight of the fruit increases only during the twelve-hour night period.

DOBSON, WILLIAM J., Mississippi State College, State College, Miss. *The effects of phosphorus starvation on the nucleic acids of Tradescantia virginiana.*—Biochemists generally agree that phosphoric acid acts as the linking agent in bonding the side chains onto the backbone of the thymonucleic and ribonucleic acid molecules. These acids can be studied cytologically by precise chemical staining and enzymes. Three groups of *T. virginiana* seedlings were cultured by three nutrient solutions containing an excess, normal, or deficient amount of phosphorus. Root-tips were processed in Navashin's Fixative and Feulgen's Stain. The ovules were fixed in Helly's Fluid and either before or after treatment with ribonuclease enzyme were processed by Unna's Technique. The nuclei of the root-tips from the excess phosphorus plants were colored the strongest by Feulgen's Stain. The controls had an intermediate amount of stain and the nuclei from the deficient plants absorbed the least amount of coloration. This gradation in stain chemically showed that the greatest amount of thymonucleic acid was present in the excess phosphorus nuclei and that the least amount was present in the deficient nuclei which were fewer, smaller, and elongate as compared with the controls. The ovules from the deficient plants stained by Unna's Technique combined with ribonuclease treatment show less evidence of phosphorus starvation than do the corresponding root-tips and the differences are not as extremely marked in the amount of ribonucleic acid present. It is believed that the lack of phosphorus prevented the formation of the nucleic acids in the starved plants.

EYSTER, H. C., Charles F. Kettering Foundation for the Study of Chlorophyll and Photosynthesis, Yellow Springs, Ohio. *The formation of starch from carbohydrates in plants as a process apart from photosynthesis.*—This is a broad study of the transformation of different carbohydrates into starch in etiolated plants and in starch-free chlorophyll con-

taining plants of several different species including corn, bean, Jimson weed, Elodea, etc. The study also includes the influence of temperature, vitamins, auxins, poisons, etc., on the starch forming process.

FRAZIER, JOHN C., Kansas Agricultural Experiment Station, Manhattan, Kan. *Regeneration in field bindweed and other noxious perennial weeds.*—The role of adventitious buds formed on the roots of the plant in the regeneration of shoots of herbaceous perennial weeds has long been recognized, having been reported by Malpighi late in the 17th century. Studies on field bindweed (*Convolvulus arvensis*), hoary cress (*Lepidium draba*), Russian knapweed (*Centaurea picris*), dogbane (*Apocynum cannabinum*), and climbing milkweed (*Gonolobus laevis*), indicate that the rapidity with which these buds give rise to new shoots has a marked influence on the optimum time interval between cultivations and hence the number of cultivations required to exhaust the root reserves of the weed in question.

Regeneration of new plants of field bindweed from segments of established plants which were buried in the soil was observed both in the field and in the greenhouse. The relation of size of segment, depth at which the segment was placed, and the effect of drying of the segment on regeneration of new plants were studied. Some concept of the maximum capacity of field bindweed to regenerate new plants was obtained especially under optimum conditions in the greenhouse.

FRENCH, C. S., AND A. S. HOLT, University of Minnesota, Minneapolis 14, Minn. *The evolution of oxygen with the simultaneous reduction of a dye by illuminated suspensions of chloroplasts.*—In the study of various hydrogen acceptors that can be used by illuminated chloroplast suspensions for the decomposition of water leading to oxygen evolution it was found that several dyes would perform this function while being themselves decolorized during the reaction. Thus a simple but indirect method of measuring this effect which is analogous to the Hill reaction has been developed. The time is determined which is required to completely decolorize, in bright light at 20°C., 5 cc. of 5×10^{-6} M. phenol indophenol at pH 6.5 in a suspension of chloroplasts, or their fragments, containing 0.15 mg. of chlorophyll. The rate of atmospheric reoxidation of this dye is too slow to cause complications in the 1 to 5 minutes required by a fresh chloroplast preparation of high activity. The method is indirect in that the disappearance of the substrate rather than the formation of oxygen is observed. In this way the activities of chloroplast suspensions have been followed at various temperatures, light intensities, and pH values. This reaction, which is now amenable to investigation in plant extracts, is influenced by these variables in much the same manner as is photosynthesis thus supporting the concept of this reaction being closely analogous to the normal evolution of oxygen by intact plants. Chloroplast suspensions have been treated with supersonic vibration and clarified by filtration or centrifugation. These preparations were then separated into various fractions by high speed centrifugation, by pH change, by adding salts or by adsorption and the activities of the different fractions compared. The activities, for a given chlorophyll content, of any of these different fractions have not yet been found to be more than two or three times greater than the activity of the starting material.

GALSTON, ARTHUR W., Osborn Botanical Laboratory, Yale University, New Haven, Conn. *The effect of a new auxin inhibitor on the flowering of Peking soybeans.*—Vegetative and protoinduced Peking soybean plants were sprayed with .0004M 2,3,5-triiodobenzoic acid (TIBA). After several hours, marked morphological responses were evident, and within 3 days the following effects were apparent: epinasty of young leaves, shortening of internodes near the apex, loss of apical dominance, darkening of the terminal bud and abscission of young leaves and buds. No vegetative plants were induced to flower by TIBA, but photoinduced plants showed a tenfold increase in the number of flower buds formed. Since many of the symptoms suggested auxin aberrations, experiments were set up to determine the effect of TIBA in the *Avena* test. TIBA itself possesses no auxin activity, but, when introduced into an agar block with indoleacetic acid (IAA) it will inhibit the activity of the latter. When TIBA is present in great molar excess, it will completely negate the effect of applied IAA. Plants in which TIBA was applied by spraying, intracotyledonary injection or application to soil showed a dry weight increment 72 per cent of the control value. However, increase in length was only 37 per cent the value of the control plants. This indicates that IAA or possibly some other growth factor is inactivated within the soybean plant by TIBA. It is suggested that reduction in auxin level is one normal metabolic accompaniment of floral initiation.

HAXO, FRANCIS, Hopkins Marine Station, Stanford University, Calif. *The carotenoid pigments of Neurospora.*—Using chromatographic procedures, the orange coloring matter of *Neurospora crassa* ("wild type") was found to consist of a mixture of carotenoid pigments. Present as components of this mixture are four well characterized carotenoids: spirilloxanthin, lycopene, gamma-carotene and small amounts of beta-carotene. Identification was based on spectral properties, adsorption characteristics, mixed chromatograms and, in the case of the first three pigments, on the nature of crystalline preparations. An apparently unique C_{40}-polyene hydrocarbon was also isolated. This pigment, for which the name neurosporene is proposed, is adsorbed on a $Ca(OH)_2$ column as a lemon yellow zone between lycopene and gamma-carotene. The spectral properties of this compound (visual maxima: 470, 441.5 mμ in petroleum ether; 502.5, 470.5, 439.5 mμ in CS_2) are markedly similar to those of 5,6-dihydro-alpha-carotene, but a melting point of 123.8°C. and a mixed chromatogram with an au-

thentic sample rule out identity. The results of an elementary analysis and a molecular weight determination suggest the possible formula $C_{40}H_{58} \pm 2H$ for neurosporene. At least one unidentified acidic carotenoid is also present in the pigment mixture. In collaboration with Dr. L. Zechmeister at the California Institute of Technology the colorless, but strongly fluorescing C_{40}-polyene, phytofluene, was identified as a normal component of the pigment extracts from *Neurospora*. The biosynthesis of all non-acidic carotenoids except neurosporene appears to be stimulated by light.

HAXO, FRANCIS, AND L. R. BLINKS, Hopkins Marine Station, Stanford University, Calif. *Photosynthetic action spectra in red algae.*—Thin thalli of marine algae were radiated with mercury lines or bands of equal energy (below photosynthetic saturation). Oxygen production was measured by a modification of the polarographic method, the tissue being in direct contact with a platinum cathode, polarized at 0.5 volt: current flow is proportional to the oxygen content of the sea water. Corrected for equal numbers of incident quanta at the wave lengths indicated, O_2 production rates were as follows (that in red light being taken as 100 per cent):

	435.8 mμ (blue)	546 mμ (green)	620–650 mμ (red)
Schizymenia (red alga)..	48% to 53%	288% to 340%	100%
Ulva (green alga).....	94%	46%	100%

The response of the red alga is thus almost complementary to that of the green, corresponding with the main absorption regions of phycoerythrin and chlorophyll, respectively. More detailed curves, taken with 8 to 15 mμ bands from a grating monochromator, show *Porphyra Nereocystis* (a bright red alga) to have a maximum O_2 production rate in the yellow green (550 mμ) falling off rapidly to a minimum in the blue violet (410 to 450 mμ), and to almost zero in the far red (700 mμ). *P. naiadum* (a more purple species) has a maximum displaced toward the yellow (580 mμ), possibly due to its higher content of phycocyanin. Importance of the "phycobilin" pigments in photosynthesis is thus strongly indicated.

HEWITT, SAM P., Cornell University, Ithaca, N. Y. *Effects of temperature on loss of dry matter and carbohydrates from leaves by respiration and translocation.*—Parallel series of *Phaseolus* and other plants were subjected over night periods of 12 to 13 hours to temperatures of 4°C., 10°C., 20°C., 30°C., 40°C. to determine losses by respiration and translocation. The matched leaf technique was used to determine losses in dry weight. The carbohydrate was extracted and analyzed. The data indicate a progressive increase in loss in dry weight over the range of temperatures up to 40°. Losses due to translocation occur up to 30°, then rapidly fall off as the temperature is increased to 40°. Analysis of the carbohydrate content shows the maximum loss due to translocation to be between 20°C. and 30°C., probably closer to 20°. Losses in dry weight, and carbohydrate due to respiration continue to rise over the entire range. Losses of dry weight are much higher than accountable by losses in carbohydrate.

HILDEBRANDT, ALBERT C., AND A. J. RIKER, Department of Plant Pathology, University of Wisconsin, Madison, Wis. *Influence of some natural and synthetic growth substances on growth in vitro of excised tobacco and sunflower tissue cultures.*—Tissue cultures of tobacco (*Nicotiana glauca* × *N. langsdorffii*) and sunflower (*Helianthus annuus* var. Giant Russian) galls were incubated on synthetic basal media lacking or containing concentrations from 1×10^{-1} to 1×10^{-13} grams per liter of one of the following compounds: cysteine hydrochloride, indole-3-acetic acid, indole-butyric acid, para-chlorophenoxy-acetic acid, 2,4-dichlorophenoxy-acetic acid, sodium 2,4-dichlorophenoxy-acetate, 2,4-dichlorophenoxy-butyric acid, alpha-naphthalene-acetic acid, alpha-naphthalene-acetamide, or beta-naphthoxy-acetic acid. With sunflower tissue cultures growth increased at low concentrations (1×10^{-9} to 1×10^{-11} grams per liter) of para-chlorophenoxy-acetic acid, and sodium 2,4-dichlorophenoxy acetate, but growth was reduced or stopped at higher concentrations (1×10^{-5} to 1×10^{-1} grams per liter). Cysteine hydrochloride had little effect on growth at any concentration used. With other compounds little effect or slightly increased growth appeared at low concentrations, but growth was reduced or stopped at higher concentrations (1×10^{-5} to 1×10^{-1} grams per liter). With tobacco tissue cultures, growth with all of the compounds was unchanged or slightly increased at low concentrations (1×10^{-13} to 1×10^{-5} grams per liter) but was inhibited or stopped at the higher concentrations (1×10^{-3} to 1×10^{-1} grams per liter).

HOLT, A. S., AND C. S. FRENCH, University of Minnesota, Minneapolis 14, Minn. *The evolution of oxygen by isolated chloroplasts immersed in solutions of various oxidizing agents.*—Oxygen evolution of such a magnitude as to be measurable by the Warburg manometric technique has been demonstrated in the past from isolated chloroplasts illuminated in the presence of such oxidants as quinone and potassium ferricyanide. A further survey has been made with a number of other possible oxidants. Of those tried, oxygen evolution was observed manometrically when chromate, indophenol or o-cresol indophenol were used. Gas evolution has been observed also from chloroplast suspensions in solutions of sodium metavanadate. In the case of the above mentioned oxidation-reduction indicators, the dyes are reduced to the leuco form, a fact which can be used to advantage for a colorimetric method of measuring the reaction velocity. Reduction to the leuco form was observed with 2–6

dichlorophenol indophenol in dilute solution. Oxygen evolution was not demonstrable manometrically with this reagent because it formed a too deeply colored blue solution which prevented the light from reaching the chloroplasts. The amount of oxygen evolved is related to the amount of oxidant present in the case of indophenol according to the equation: $2\ In + 2\ H_2O \rightarrow 2\ H_2In + O_2$. In the case of chromate, on the basis of a reduction from Cr^{+6} to Cr^{+3}, the volume of oxygen evolved has been found to vary between 55 and 75 per cent of the theoretical amount. The pH maxima with ferricyanide and chromate in phosphate buffer were found to be pH 7.1 and pH 7.3 respectively.

HOPKINS, E. F., AND K. W. LOUCKS, Citrus Experiment Station, Lake Alfred, Fla. *Effect of heavy metals on susceptibility of oranges to stem-end rots.*—Although copper is a fungicide, thus far, all treatments with soluble copper compounds have increased infections in oranges by *Diplodia natalensis* and *Promopsis citri.*, especially when copper sulphate is combined with sodium thiosulphate to give cuprous copper. One such treatment resulted in 58 per cent rot in 1 week while the check showed none. The stimulating effect has been repeatedly shown even with small amounts of copper. Treated fruits become infected 1 to 3 weeks sooner than checks and after 3 weeks have several times the number of infections. No visible phytocidal action or high incidence of *Penicillium* infections, indicative of chemical injury to the fruit peel, has been observed. Immature oranges normally resistant to stem-end rot become very susceptible when treated with copper. Solutions of $Na_2S_2O_3$ cause similar effects due to solvent action on insoluble copper spray residues. In potato agar culture $CuSO_4$ at 10 ppm *Phomosis* and *Diplodia* grew normally, 500 ppm was fungistatic for *Phomopsis* but *Diplodia* was not affected and 500 ppm plus $Na_2S_2O_3$ did not affect either. In protein-free solution 500 ppm was lethal to both. No stimulating action on growth was noted and it is suggested that increased permeability of copper treated fruits may explain their greater susceptibility to infection. Mn, Zn, Fe and Al likewise increased the percentage of rots but not so markedly as Cu. Zn and Fe gave a four-fold increase over the checks. In contrast Ca and Mg caused no significant change.

JACKSON, J. R., Alabama Polytechnic Institute, Auburn, Ala. *Response of Cyperus rotundus to 2,4-dichlorophenoxyacetic acid.*—Results of attempts to control nut grass by the use of sprays of 2,4-dichlorophenoxyacetic acid and its esters suggest that this plant might be useful in testing the efficacy of herbicides. Leaves and shoots were killed. Tubers were killed or their germination was inhibited in ratio to their proximity to aerial structures.

KELLY, SALLY M., Brooklyn Botanic Garden, Brooklyn, N. Y. *Further studies on aerobic respiration and water uptake in Avena coleoptile segments.*—As determined by increase in fresh weight, water uptake in Avena coleoptile segments is an aerobic process. The stimulation of water uptake by indoleacetic acid is also an aerobic process. Water uptake of segments soaked 6 or more hours in buffered sucrose solutions or in distilled water was prevented when nitrogen was bubbled through the solutions continuously. Upon the addition of indoleacetic acid in concentrations of 1 to 40 mg./l., the water uptake of segments in aerated solutions was stimulated 50–70 per cent; no such stimulation occurred when nitrogen was bubbled through the indoleacetic acid solutions. Further evidence of the aerobic nature of the process is the prevention not only of water uptake but of the auxin stimulation of water uptake by substances which also inhibit aerobic respiration.

KING, GLADYS S., Southern Regional Research Laboratory, New Orleans, La. *2,4-D herbicides for water hyacinths.*—Although applications of 2,4-D to water hyacinths effectively kill upright petioles and roots of treated plants, about 50 per cent of these plants, apparently in a particular phase of development, replace themselves through a rapid formation of new plant parts from basal buds. This effect was diagnosed as an apical dominance phenomenon. As upright petioles are killed by 2,4-D, the upward movement of substances required with auxin for growth ceases, and these along with auxin are diverted to basal buds (Went, Plant Physiol. 13, 55, 1938). The auxin combines with the enzyme and substrate substances necessary for a growth reaction (Skoog. *et al.*, Amer. Jour. Bot. 29, 568, 1946), and buds which give rise to the replacement plants, develop. On this basis, compounds were added to 2,4-D which would be competitive with auxin in its combination reaction including phenyl acetic acid, trans-cinnamic acid, and indole butyric acid. Other additions were made of highly active auxin compounds, indole acetic acid, indole propionic acid, and naphthalene acetic acid, to introduce competition among auxin molecules themselves in the combination reaction. A third method of inhibiting this 2,4-D influenced basal bud growth was the addition of various forms of K,N, and P (Skoog, Amer. Jour. Bot. 31, 19, 1944, and unpublished). Any of these compounds used in combination with 2,4-D completely kill water hyacinths. Large scale tests now under way have shown that of these possible combinations, that of phenyl acetic acid with 2,4-D is the most practical for commercial use. This same herbicide formula might be effective for other weeds with similar replacement tendencies after 2,4-D treatment.

LANG, ELAINE, Cornell University, Ithaca, N. Y. *The effects of ultra-violet radiation on the viability of spores of Neurospora.*—Viable conidia of *Neurospora* cultures were produced at temperatures between 15°C. and 36°C. With the addition of biotin and sugar to Fries solution, an increase in germination of the macroconidia was noted. Increased tolerance of macroconidia to ultra-violet rays was observed when biotin was added to Fries solution. A

definite protective effect was also evident when 1 per cent sugar was added to Fries solution. The type SB sterilamp which has been used by other workers to produce mutations was utilized throughout this experiment. The LD 50 lethal point was determined to be 45 seconds at 5.47 cm. and 5 minutes at 30 cm.

LIVINGSTON, L. G., AND GRACE MEDES, Swarthmore College, Swarthmore, Penn., and Lankenau Hospital Research Institute, Philadelphia, Penn. *Biosynthesis of C^{13} compounds. I. The biosynthesis of C^{13} labeled starch.*—Since many compounds of potential value for intermediary metabolism studies are difficult or impossible to synthesize by known organic chemical procedures, an attempt is being made to devise effective means of biosynthesis, using $C^{13}O_2$ as a starting material, and the photosynthetic process in green plants. The purified products isolated from the plant material can be used directly in experimental work, or as a source of more complex radicals for compounds synthesized chemically or biologically. In the experiment described here, an attempt was made to devise a specific biosynthetic scheme capable of producing a high yield of C^{13} labeled starch with a minimum of dilution by C^{12} compounds already present in the plant. The approach is based on the fact that the excess carbohydrate formed in a leaf carrying on photosynthesis at a high rate is temporarily stored as starch in the chloroplast in which it is produced. Mature, detached, starch depleted leaves of *Phaseolus vulgaris* were used. The leaves were arranged around the inner periphery of a closed illuminated, water cooled chamber. They were supplied with an atmosphere containing 11.2 per cent CO_2 (53.86 millimoles); the CO_2 having a C^{13} content of 8.36 per cent. In 48 hours, the concentration of CO_2 was reduced below 0.5 per cent, at which time the experiment was terminated. The leaves were fractionated, and the C^{13} content of each fraction was determined. The C^{13} content of the starch was 8.15 per cent, indicating that very little dilution occurred. The starch yield accounts for 66 per cent of the total carbon introduced into the photosynthetic chamber as CO_2.

MYERS, JACK, University of Texas, Austin, Tex. *Oxidative assimilation in relation to photosynthesis in Chlorella.*—An oxidative assimilation of acetic acid and glucose in darkness is demonstrated in the green alga, *Chlorella pyrenoidosa.* From manometric experiments it can be shown that one mol (CH_2O) per mol acetic acid and five mols (CH_2O) per mol glucose are produced. The time required for complete utilization of a limited amount of acetic acid or glucose is not affected by illumination in the absence of carbon dioxide. The time required for complete utilization of a limited amount of glucose is not affected by the simultaneous occurrence of photosynthesis. It must therefore be concluded that the accumulating product of photosynthesis cannot be glucose but must be some slowly respirable (storage) material. Possible further relationships between oxidative assimilation and photosynthesis may be followed by studying, in darkness and in light, the time course of oxidative assimilation of substances which are possible intermediates in the two processes.

PRATT, HARLAN K., AND ROY E. YOUNG, University of California, Division of Truck Crops, Davis, and Division of Horticulture, Los Angeles, Calif. *The identification of ethylene as a volatile product of ripening avocados.*—A constant air stream was passed through large jars containing Nabal avocados and thence through an absorption apparatus containing bromine in a solvent (methylene chloride). After six days, the solution from the absorber was fractionated under reduced pressure leaving 700 mg. of clear yellow oil, presumably ethylene bromide. This was reacted with aniline to produce the crystalline derivative, N, N′-diphenylethylenediamine. The identity of this product was established by its melting point and that of a mixture with an authentic sample.

SHALUCHA, BARBARA, Brooklyn Botanic Garden, Brooklyn, N. Y. *Auxin and nitrogen content of developing peach shoots.*—A study of the seasonal auxin and nitrogen content in developing shoots of peach (*Prunus persica,* var. South Haven), showed no direct correlation between the auxin and nitrogen. Auxin content of tissues *was* correlated, however, with cultural conditions under which the trees were growing. Maximum auxin yields and maximum vegetative growth were obtained from shoots of trees growing under sod with straw mulch. In general, auxin yields were proportional to the rate of vegetative growth. At the end of the growing season, auxin content was similar for all trees regardless of cultural conditions. Auxin yields and nitrogen content of shoots were significantly reduced with initial development of the fruit embryo independent of cultural conditions; however, auxin yields increased again as the embryo matured.

TANG, Y. W., AND JAMES BONNER, California Institute of Technology, Pasadena, Calif. *Enzymatic inactivation of indole acetic acid.*—An enzyme system which causes inactivation of indole acetic acid is contained in etiolated pea epicotyls and in other tissues. The enzyme is readily purified, contains no dialyzable coenzyme, and appears to be an iron protein. It attacks indole acetic acid via the side chain in the 3 position with the liberation of indole. Oxygen is essential to action of the enzyme and oxygen is consumed in the reaction. The enzyme shows great substrate specificity and does not attack compounds other than indole acetic acid in so far as tests have been carried out.

WHITAKER, ELLIS H., State Teachers College, Oneonta, N. Y., and Cornell University, Ithaca, N. Y. *A study of the exudate of Drosera longifolia L.*—Samples of the exudate of *Drosera longifolia* L. have been collected by a centrifugation technique and investigated with regard to certain physical and chemical properties. When dried *in vacuo,* or at 50°C., the dry weight of the exudate gives values

ranging from 1.06 per cent to 1.20 per cent of the fresh weight. The specific gravity of fresh exudate is from 1.0065 to 1.0080, and its pH ranges from 4.12 to 4.17. The mean index of refraction, determined at 25.5°C., is 1.3353, with a mean dispersion value of 0.009. The osmotic concentration, determined by the freezing point method, is 0.107 M. The reducing sugar content of fresh exudate, expressed as glucose, is 1.377 mg./ml. Working with dried exudate, the total nitrogen content is 20.0 mg./g.; the nitrate nitrogen, 2.2 mg./g.; the total alpha-amino nitrogen, 6.1 mg./g., and the non-diffusible alpha-amino nitrogen 3.4 mg./g. An investigation of the proteolytic activity of fresh exudate gave positive results on both egg-white and hemoglobin substrates, with optimal pH values at approximately 2.0 and 4.2, respectively. An investigation of the corresponding properties of exudate from plants of *Drosera filiformis* Raf., grown under aseptic conditions, is currently being undertaken.

WILDMAN, SAM, AND JAMES BONNER, California Institute of Technology, Pasadena, Calif. *Separation and properties of the auxin protein of spinach leaves.*—Methods have been devised for the large scale extraction of the protoplasmic contents of the cells of spinach leaves. The time between extraction and drying in a lyophil apparatus is short and all processing is done at 4°C. The leaves are very finely ground in a Charlotte colloid mill and the protoplasmic juice is separated from cell walls and unbroken cells by centrifuging in a basket centrifuge lined with sharpskin filter paper. More leaves are added to the centrifuged juice and the process continually repeated. It is possible, by this means, to produce an extract containing more than 6 per cent solid matter. The juice is immediately frozen and dried in the frozen condition. When dry, it can be stored for long periods without evident deterioration in enzymatic activity. It is estimated that more than 60 per cent of the total leaf nitrogen is extracted by this process. Two methods have been used to separate chloroplastic material from the cytoplasm: (1) lyophilized material is dispersed into a neutral, 0.25 saturated $(NH_4)_2SO_4$ solution and centrifuged for 1 hour at 4,000 r.p.m. to cause the precipitation of the chloroplastic material; (2) lyophilized material is dispersed into ice water and centrifuged at 18,000 r.p.m. for 1 hour causing the deposition of the chloroplastic material. In both cases, enough starting material was used to give a concentration of 20 per cent solids. The cytoplasmic proteins were separated by adding enough neutral, saturated $(NH_4)_2SO_4$ to the supernatants obtained by centrifuging to give a 0.35 saturated solution. The precipitated protein was removed by centrifuging at 4,000 r.p.m. for 1 hour and completely dissolved in a buffer more alkaline than pH 6.2. Two reprecipitations with 0.33 saturated $(NH_4)_2SO_4$ yielded an electrophoretically homogeneous product designated Fraction I. A small amount of Fraction I remained in solution in 0.35 saturated $(NH_4)_2SO_4$ but could be precipitated by concentration in moving air followed by long dialysis against distilled water. Fraction I precipitated by dialysis could also be dissolved in a buffer. The proteins which remained in solution after dialysis were designated Fraction II. Hydrolysis of Fraction I with hot alkali or proteolytic enzymes yielded auxin. Auxin was not removed from the protein with cold acid, cold alkali, prolonged dialysis against water or buffer, or by electrodialysis. Fraction I appeared to have the exclusive enzymatic properties of a phosphatase which could rapidly hydrolyze ATP, creatine phosphate, fructose 1–6 diphosphate, β-glycerophosphate, and phytic acid. The enzyme was apparently unable to cause the transfer of high energy phosphate from ATP to substrates containing the guanido group. It was not possible to detect the phosphorylation of sugar in the presence of the enzyme and ATP. Fraction I constitutes about 75 per cent of the total cytoplasmic proteins. Osmotic pressure measurements indicate a molecular weight of 185,000. The protein contains pentose but no P. The protein has an absorption maximum at 280 mμ, has a brownish-red coloration but no maxima in the visible spectrum. The protein rapidly precipitates at pH values more acid than 6 and the precipitation is partially irreversible. Salt is required to keep the protein in solution. Auxin is not found in Fraction II. When sufficiently concentrated (1.5 per cent), Fraction II displays five electrophoretically distinguishable components. When completely free from Fraction I, it is blue green in color and exhibits a broad absorption maximum in the region of 570–590 mμ. A variety of enzymatic activities can be shown to be associated with non-auxin containing Fraction II. So far, four dehydrogenases, catalase, peroxidase, polyphenoloxidase and tryptophan-converting enzyme have been found in this preparation.

WILDMAN, S. G., MARIO G. FERRI, AND JAMES BONNER, California Institute of Technology, Pasadena, Calif. *The enzymatic conversion of tryptophan to auxin by spinach leaves.*—Evidence is presented that spinach leaves are capable of rapidly converting tryptophan to a plant growth substance. In 3.5 hours, ten times more auxin was extracted from leaves infiltrated with tryptophan and neutral buffer than with buffer alone. The reaction was also found to occur in lyophilized protein preparations, with the enzyme system being confined to the cytoplasm of the leaf cells. Oxygen was required for the enzymatic conversion of tryptophan to auxin. The reaction was completely inhibited by 10^{-3} M sodium bisulfite and 10^{-3} M cyanide suggesting that a carbonyl group is formed during the conversion of tryptophan. When indole pyruvic acid is infiltrated into living leaves, a large increase in auxin activity can be demonstrated suggesting that this compound is the keto intermediate in the conversion mechanism. However, cell-free extracts are unable to convert the compound, possibly because of the rapidity with which the compound polymerizes in

solutions more alkaline than pH 4.0. Tryptamine and indole acetaldehyde were not converted to auxin by spinach leaves or extracts. The hypothesis is presented that tryptophan is the principal precursor of auxin and indole acetic acid the principal auxin in spinach leaves. Because of the dynamic equilibrium existing between proteins and amino acids, it is considered that there is always a readily available supply of tryptophan present for conversion to auxin, and in this sense, auxin *per se* is probably never limiting in the growth reaction. It is believed that before auxin can perform in growth, it must first combine with a protein to form a specific enzyme. According to this scheme, ether-extractable and diffusible auxin is auxin formed from tryptophan but not yet combined with protein. The auxin-protein could also act as a potential source of "free" auxin if the "bound" auxin were released by the action of proteolytic enzymes, hydrolysis with alkali, or slow autolysis of the tissues in the presence of wet ether. Such treatments result in the destruction of a vital enzyme and probably have no resemblance to the normal formation of auxin using tryptophan as the precursor.

SYSTEMATIC SECTION

(Abstracts of this Section are arranged alphabetically according to author)

BEETLE, ALAN A., University of Wyoming, Laramie, Wyo. *Statistical analysis of the genus Scirpus, Cyperaceae—a warning.*—This paper is a statistical analysis of 1638 names in *Scirpus* as to place and date of publication, authorship, validity, generic disposition, and similar matters—or a review of what a monographer undertaking work in a large genus can expect.

BUTTERS, F. K., AND R. M. TRYON, JR., University of Minnesota, Minneapolis, Minn. *A fertile hybrid Woodsia.*—Part of a single frond of a plant of *X W. Abbeae* developed viable spores. Young sporophytes were produced but died at the end of a few months. A comparative study of the size of the annulus cells between the fertile and the sterile parts of the frond indicate that the former was tetraploid.

CHIN, T. C., AND HEBER W. YOUNGKEN, Harvard University, Cambridge, and Massachusetts College of Pharmacy, Boston, Mass. *The cytotaxonomy of Rheum.*—The genus of *Rheum* includes species of several taxonomic groups. The numbers of chromosomes were found to be $2n = 22$ for *Rh. franzenbachii* Muent., $2n = 44$ for *Rh. tartaricum* L., *Rh. compactum* L. and *Rh. altaicum* L. and $2n = 66$ for *Rh. australis*. The size of pollen and the stature of the plants are associated with the chromosome numbers. However, *Rh. emodi* Wall. possessing $2n = 22$ has larger pollen grains and chromosomes in comparison with *Rh. franzenbachii* Muent. These and the early reports on the different size of chromosomes between *Rh. speciforme* Royle and *Rh. palmatum* L. together with the chromosome association in *Rh. australis* suggest the existence of two genoms of 11 chromosomes and that the present-day tetraploid species arose by amphidiploidy.

CLAUSEN, ROBERT T., CHARLES H. UHL, AND GILY E. BARD, Cornell University, Ithaca, N. Y. *The natural history of Sedum Nevii and S. glaucophyllum.*—*Sedum Nevii,* as described by Asa Gray, was an aggregate of two biological species: nomenclaturally typical *S. Nevii* with a gametic number of six chromosomes at the extreme southern end of the Appalachian Highlands and *S. glaucophyllum* with a gametic number of fourteen or twenty-eight chromosomes in the central Appalachian Highlands from the Roanoke to the Potomac Valleys on the eastern side and on the west primarily in the headwaters of the Kanawha River. *S. Nevii* and *S. glaucophyllum,* though cytologically distinct, are only slightly different morphologically. The leaves of *S. glaucophyllum,* besides being somewhat glaucous, are usually broader and longer and in denser rosettes. Anyone familiar with the species can readily separate living plants, but herbarium specimens are sometimes difficult to identify. *S. glaucophyllum* has an altitudinal range from 60 to 1067 meters. Though it occurs on a variety of rocks—sandstone, limestone, granite, gabro and gneiss, calcium seems to be a limiting factor, since all rocks on which it has been found have given a positive reaction for calcium carbonate. Likewise, *S. Nevii* seems to be limited by the presence of calcium. The two populations of *S. Nevii,* in the valleys of the Black Warrior and Cahaba Rivers respectively, differ markedly in foliage and habit. These differences are maintained when plants of the two types are grown under uniform conditions. These populations, geographically isolated since the area between the two valleys is lacking in suitable habitats, seem to have the characteristics of taxonomic subspecies.

CRONQUIST, ARTHUR, Department of Botany, University of Georgia. *A revival of the genus Petradoria Greene.*—In 1895 E. L. Greene proposed the monotypic genus *Petradoria,* based on *Chrysoma pumila* Nutt. *Chrysoma pumila* Nutt. had been transferred to *Solidago* by Torrey and Gray, although the name *S. pumila* was preoccupied. Greene's segregation has not been generally accepted, and S. F. Blake has proposed the name *Solidago petradoria* to replace the illegitimate combination *S. pumila* (Nutt.) T. & G. A notable exception to the general reduction of *Petradoria* by conservative taxonomists was the stand taken by Hall and Clements, who considered it a distinct genus allied to *Haplopappus* and *Chrysothamnus.* Their comments on the genus were buried in the introduction to their revision of *Chrysothamnus,*

and have perhaps not been generally noted. The type of root system in *Petradoria* shows its close relationship to *Haplopappus,* from which it differs in its sterile disk-flowers and vertically aligned phyllaries. The vertical alignment of the phyllaries is suggestive of *Chrysothamnus,* from which *Petradoria* differs in its sterile disk-flowers, the presence of pistillate flowers, and its herbaceous rather than shrubby habit. Although it is monotypic, *Petradoria* cannot properly be included in any other genus. The position taken by Hall and Clements, that it is a distinct genus allied to *Haplopappus* and *Chrysothamnus,* is here upheld.

DUNCAN, WILBUR H., Department of Botany, University of Georgia. *New and unusual collections from Georgia.*—An interesting and unusual habitat for Georgia, a mountain bog in the northeastern part of the state, was visited six times during 1946 at intervals of about a month. Several species were found that are apparently new records for the state. They include *Kalmia angustifolia* L., *Cypripedium acaule* Ait., *Helonias bullata* L., and *Triantha glutinosa* (Michx.) Baker. *Sarracenia purpurea* L. is also abundant there. Although this is probably its most southern distribution in a mountain bog, it is also found rarely in coastal plain bogs farther south. A second area of particular interest in northeastern Georgia is located west of the Appalachian Trail just south of the North Carolina state line in the region of Hightower Bald. Here in a rich mountain cove are several trees of *Cladrastis lutea* K. Koch, the Yellowwood tree, a new record for the state. *Liparis liliifolia* (L.) L. C. Rich and *Muhlenbergia tenuiflora* (Willd.) B.S.P., which are probably new records, were found associated with the *Cladrastis.* Several new collections for the state were found on the upper, north slopes of Hightower Bald. They include *Sambucus pubens* Michx., *Viburnum lantanoides* Michx., *Acer spicatum* Lam., and *Allium tricoccum* Ait. Other new records include: from the mountains, *Vaccinium hirsutum* Buckley in Dawson Co. and *Aster chlorolepis* Burgess in Murray Co.; and from the coastal plain, *Crataegus brachyacantha* E. & S., previously not known from east to Louisiana. Most of the species listed represent the southern-most record for the species concerned. After driving 60,000 miles while doing field work in Georgia since 1939, I feel that it would be well to report the apparent absence of certain species which are reported by many manuals as occurring in Georgia, especially, *Quercus imbricaria* Michx. and *Picea rubra* Link.

FLINT, LEWIS H., Louisiana State University, Baton Rouge, La. *Studies of freshwater red algae.*—Progress in the collections and identification of plants in this group may be noted with the following: *Sirodotia tenuissima* (Holden) Skuja, *Batrachospermum radians* Sirod., *Batrachospermum helminthosum* Sirod., *Batrachospermum Crouanianum* Sirod., *Batrachospermum coerulescens* Sirod., *Batrachospermum virgatum* Sirod., *B. viride* Sirod., *Tuomeya fluviatilis* Harv.

FOSBERG, F. R., Washington, D. C. *Botanical aspects of the U. S. C. C. economic survey of Micronesia.*—The botanical personnel of the Economic Survey sent to Micronesia from May to August, 1946, were E. Y. Hosaka and F. R. Fosberg, with Y. C. Wong as assistant. All of the major islands and some of the small ones in the former Japanese Mandate were visited. Some time was spent on Guam. Collections and observations on the vegetation and the economic plants were made, the collections totalling 3750 numbers. This material will provide a better basis than has previously been available for an understanding of the Micronesian flora.

GOODMAN, GEORGE J., University of Oklahoma, Norman, Okla. *The relationships of the flora of the Luka Chukai mountains, Arizona.*—The Luka Chukai Mountains constitute a small range in northeastern Arizona at the north end of the Chuska Mountains. The highest points are about 9500 feet in elevation. Surrounded on all sides by desert, the range is well isolated, especially from the central and northern Rocky Mountain type of flora. Nevertheless, a tabulation of the results of parts of two summers' collecting indicates that 60 per cent of the flora is of the Rocky Mountains. Many of these species are known in Arizona only from its northern part, or indeed from the Luka Chukais. Very few species are of wide general distribution. The next largest group, about 30 per cent, are of species whose central distribution is near the region studied. The smallest group consists of those southern and Mexican plants whose northern-most extension is the Luka Chukai Mountains.

HOWARD, RICHARD A., Harvard University, Cambridge, Mass. *Report on survival training in the AAF.*—To meet the needs of operational groups in the South Pacific war theaters, special men of the AAF were trained to become survival instructors to flying personnel. Their job was to introduce, to men who might need it, principles of emergency survival, the ways and means of finding food, water, direction, shelter, protection, and rescue. The training program for these instructors was limited to a three weeks period. New methods of presenting biological information were sought and tested in lectures and in practice. The results of this training program suggest new "survival hints" for field biologists as well as methods of teaching general biology or even advanced classes in taxonomy. Material was presented by conventional movies and slides, books and printed materials, and by unconventional lectures and demonstrations and by actual testing of achievement and ideas in the field. Besides the desired results of reducing casualties, the following results also are evidenced: (1) The increase in individual activity in an area when the men are confident of their ability to care for themselves. The ability to find food and water are still the survival factors of most concern to the individual. This is applicable to soldier, camper, or field botanist. (2) The ability of the novice to learn

quickly and to remember many plants if these are presented to him in classes. This is particularly true of recognition of edible and poisonous plants. (3) Introduction to the natives of an area of new ways to use the local plants and plant products, as well as the introduction of new fruits and vegetables.

HUGHES, ELWYN O., University of Oklahoma, Norman, Okla. *New and rare fresh-water algae from Nova Scotia.*—The fresh-water algal flora of Nova Scotia remained practically unknown until 1939 when M. W. Smith listed 83 species from a small lake in Yarmouth County. Four previous workers (Joshua '85, Turner '85, Borge '09, and Roscoe '35) had recorded a total of 29 species in the province. Collections made by the author and friends from 1936 to 1943 have yielded up to the present over 300 species, the majority of which are new records for the area. One new genus and five new species of Chlorophyceae have been found in the Medway River Valley of southwestern Nova Scotia. The new genus in its vegetative characteristics resembles members of the Ulotricaceae and Zygnemataceae. Since evidence of sexual or asexual reproduction is lacking, the position of the genus within the Chlorophyceae is obscure. The new species (three in *Bulbochaete,* one *Oedogonium,* and one *Spirogyra*) have recurred in subsequent summers in the same habitat. The new genus, however, was found only in 1941, despite further collecting in 1942 and 1943. Among Nova Scotian species previously reported only once in the literature are *Chaetonema ornata* Transeau (Alabama), *Bulbochaete suberecta* (Collins) Tiffany, (Massachusetts), *B. Woronichini* Tiffany (Tiflis, Caucasus), and *Spirogyra minutifossa* Jao (Massachusetts).

LEWIS, HARLAN, University of California, Los Angeles, Calif. *Polyploidy in the Californian Delphiniums.*—Three native species of *Delphinium* (larkspur) have been found to consist of both diploid and tetraploid races: *D. Hanseni, D. gypsophilum* and *D. variegatum.* Their distributions are centered about the Great Valley of California. *D. Hanseni* and *D. variegatum* are partially sympatric. *D. Hanseni* and *D. gypsophilum* are connectant. *D. gypsophilum* and *D. variegatum,* although their distributions overlap, are ecologically separated. The tetraploid races of each are included within the ecological and geographical range of the corresponding diploids. The diploid races are roughly equal in fertility when hybridized; the tetraploid races are highly interfertile, approaching the degree of interfertility of the parent species. Natural tetraploid, triploid, and diploid hybrids have been found in nature in the areas of contact. Nevertheless, despite this degree of interfertility and the production of natural hybrids, no evidence of introgression has been found. There is no evidence that polyploidy has increased the diversity of the species concerned, save with respect to chromosome number. *D. gypsophilum* and *D. variegatum* are relatively uniform morphologically, and the tetraploid races of each are indistinguishable from the diploid. Hence, they have not been accorded nomenclatorial status. *D. Hanseni,* however, is characterized by three morphological and geographic races, of which one is tetraploid. These have been given nomenclatorial status.

MARTIN, A. C., U. S. Fish and Wildlife Service, Bowie, Md. *The slighted role of seeds in plant phylogeny.*—A comparative study on the internal morphology of seeds of 1287 genera has provided basis for re-appraising current concepts of plant phylogeny. Lines of relationship denoted by seeds have been found to diverge considerably from conventional systems founded largely on flowers and fruits. Furthermore this broad survey has also indicated that the internal characteristics of seeds are stable, reliable classification criteria. Assumptions of flexible reversibility such as implied by present systems of phylogeny are contrary to the evidences of stability. Twelve distinctive and seemingly fundamental forms of seed development are recognized for the plant kingdom. Particular importance is attached to two primary lines of seed evolution, in one of which the embryo has extended itself more or less peripherally along the inner surface of the seedcoat and exterior to a distinctly starchy endosperm. In the other major progression the embryo has, in contrast, developed axially through the core of an endosperm that is seldom starchy. Prevailing systems of plant relationship have been elaborated with a minimum of benefit of knowledge from seed evolution due largely to the fact that comparative study on the internal morphology of seeds has remained a neglected field. It will seem fitting that future taxonomic systems adapt themselves to evidence from comparative seed morphology and give it a prominent place along with evidence from flower and fruit criteria.

OGDEN, E. C., University of Maine, Orono, Me. *Use of stem-anatomy characters in identification of species of Potamogeton.*—The most useful stem-anatomy characters are: the arrangement of bundles in the stele, the type of thickening of the cells of the endodermis, and the presence or absence of bundles in the cortex. Other helpful characters include: the position of the cortical bundles, the presence or absence of a layer of cells next to the epidermis, length in proportion to width of the cells of the epidermis, and shape of the stem in cross-section. Combinations of these characters will not separate all of the forty-one species of *Potamogeton* in North America, but in many cases the species can be named with assurance from a scrap of stem only a few millimeters long. Free-hand sections, stained a second or two in ordinary ink, are satisfactory for making an anatomical examination.

PRESCOTT, G. W., Dept. of Botany, Michigan State College, East Lansing, Mich. *The alpine algae of Ecuador.*—While exploring for Cinchona in the high Andes about one hundred collections of algae were made. Although collecting was limited to incidental catches as opportunity afforded, the samples contain a large number of species and

permit some observations on the distribution of algae in high altitudes. The inter-Andean highland (9,000–10,000 ft.) is almost devoid of standing water and habitats favorable for algae exist only in the rapidly flowing streams, in seeps on embankments and cuts, and on the walls of canyons. Here in the semi-arid plateau the algal flora is almost entirely Myxophycean and terrestrial and subaerial forms predominate. Interesting collections were made in the floating bogs and small ponds among the ancient mounds of volcanic ash in the high paramos (12,000–14,000 ft.). The water in such situations, obviously more acid than in the plateau area, supports a greater variety of species, including desmids. Most of the species listed are new for the country because very few reports have been published on the algae of Ecuador. Several species are described as new.

REEDER, JOHN R., Biological Laboratories, Harvard University, Cambridge, Mass. *Additional evidences of affinities between Eragrostis and certain Chlorideae.*—Agrostologists agree that the tribe Chlorideae as commonly interpreted is a heterogeneous group. The only character that the genera have in common (other than those which place them in the subfamily Festucoideae) is that of the inflorescence. On the basis of gross morphology some of the genera seem to have close affinities with the tribe Festuceae. C. E. Hubbard and others have placed such genera as *Eleusine, Dactyloctenium, Leptochloa,* etc., in the tribe Eragrosteae, which derives its name from the genus *Eragrostis,* commonly considered a member of Festuceae. Study of sections of the embryos of several species of *Eragrostis, Eleusine, Dactyloctenium,* and *Leptochloa* shows that they have a strikingly similar structure. This anatomical evidence is in accord with that from gross morphology in indicating that the affinities of *Eleusine, Dactyloctenium,* and *Leptochloa* lie with *Eragrostis.*

ROLLINS, REED C., Stanford University, Calif. *Can populations of plants showing the combined effects of polyploidy, interspecific hybridization, sexuality, and facultative apomixis be usefully organized into systematic entities?*—Genetical and cytological studies of natural populations of *Parthenium argentatum* and *P. incanum* have shown that (a) polyploidy is common in both species, (b) the two species hybridize at several chromosome levels (c) certain phases of both species reproduce by sexual means (d) certain phases of both species reproduce by facultative apomixis. Not only do these factors operate independently to determine the nature of a given population, but frequently several factors operate simultaneously. Also, some of the present-day populations show the impact of different ones of these factors, perhaps functioning independently, but at different periods during their development. Thus characteristics developed through hybridization were subsequently fixed by apomixis; segments of a freely interbreeding population were fixed in the same manner, etc. Examples of the effects in terms of plant expression are discussed. A systematic study of *Parthenium argentatum* and *P. incanum* utilizing the cytological, genetical and morphological data available has been made. The number of seemingly constant entities below the species-level and microentities subdividing these, proved to be so great that an attempt to treat them systematically was abandoned as being not worth while. As a result, the eight varieties of *P. argentatum* created by Nicolaieff are treated as synonyms of the typical variety.

ROUSSEAU, JACQUES, Jardin Botanique, Montreal, Canada. *The botanical survey of Lake Mistassini.*—The author spent the three last summers in this district and was accompanied, in 1944, by Mr. Ernest Rouleau. The main features are as follows: (1) Complete list of the vascular flora, (2) partial survey of the bryophytes, fungi and algae, (3) new species and varieties, (4)affinities of this flora with the flora of the Rocky Mountains, Northwest Territories, Northern Section and Southern lower Anticosti, Gaspe, and Newfoundland, (5) ecologic documentation, (6) northern limit of a great number of the species, and (7) ethnobotany of the Mistassini Indians.

SVENSON, H. K., American Museum of Natural History, 79th Street, Central Park West, New York, N. Y. *The Linnaean concept of species.*—Species of Linnaeus were defined by the polynomial specific names, which the trivial names (*i.e.,* the "specific" names of common usage) represented.

TRYON, R. M., JR., AND L. E. BOOHER, University of Minnesota, Minneapolis, Minn. *Remarks on the genus Elymus in Minnesota.*—The variability of the four species *E. canadensis, E. villosus, E. interruptus* and *E. virginicus* is discussed with an evaluation of described varieties and forms. Specific characters and distribution in Minnesota are also mentioned.

VAN STEENIS, C. G. G. J., 's Lands Plantentuin, Buitenzorg, Java, Netherlands Indies. *The Flora Malesiana, a plea for the preparation of a cooperative flora for the flowering and cryptogamic plants of the Malaysian region.*—After a review of the principal publications of the past concerning the flora of the Malaysian region the scope, possibilities, and aims of a cooperative flora of Malaysia, which will include the higher as well as the lower plants, is outlined. The Flora will be prepared on an international cooperative basis and the assistance of competent monographers all over the world is being solicited. The Flora has the official and material support of the Government of the Netherlands Indies and financial aid is also being given by a number of other organizations. The speaker will stay in the Netherlands for several years to organize the *Flora Malesiana* on behalf of the Government of the Netherlands Indies, and is at present visiting the U. S. A. for this purpose.

VERDOORN, FRANS, Chronica Botanica, Waltham, Mass. *On the need for a series of generic floras,*

particularly for the cryptogamic plants of the old and new world tropics.—No one can claim to be a fair all-round biologist without knowing the principal forms of life, plant and animal, surrounding him. Yet, this knowledge is often difficult to attain, particularly for the biologist transferred to the tropics. The publications he will consult have, as a rule, been prepared by taxonomic specialists for taxonomic specialists, or they are of a popular nature and have been prepared primarily for the general public. Though a non-specialist cannot follow the taxonomic specialist in his studies of species and varieties, many a general biologist, agronomist, and intelligent amateur will follow the specialist in the realm of the genera. In generic floras and faunas of the principal genera of various regions we can easily give, in little space, all the principal forms of these regions, with enough information about ecology and biology to answer most of the questions with which our colleagues are trying to cope. If well done, such generic floras and faunas cannot fail to be a source of constant inspiration and also an important bridge over the gap between taxonomic and general biology.

JOINT SESSIONS

Joint Session with the Ecological Society of America

SHARP, AARON J., University of Tennessee, Knoxville, Tenn. *A preliminary report on some phytogeographical studies in Mexico and Guatemala.*—There are species in all plant groups which are common to eastern United States and the highlands of Mexico and Guatemala. The most of them can be found in one or more of the following areas: the eastern escarpment in Mexico between San Luis Potosí and southern Veracruz, the central highlands of Chiapas, and the Cuchumatanes plateau of Guatemala. The vascular plants noted there include: *Asplenium resiliens, Osmunda regalis, O. cinnamomea, Lycopodium clavatum, L. complanatum, Selaginella apoda, Pinus strobus, Smilax glauca, Liquidambar styraciflua, Prunus serotina, Cercis canadensis, Ptelea trifoliata, Rhus radicans, Acer negundo, Berchemia scandens, Parthenocissus quinquefolia, Ascyrum hypericoides, Cornus florida, Nyssa sylvatica, Gelsemium sempervirens, Epifagus virginiana, Mitchella repens* and others. Many exhibit a disjunct distribution. In addition there are many species in the highlands of Mexico and Guatemala closely related to plants in eastern United States.

Joint Session with the Mycological Society of America

JACKSON, H. S., AND E. R. DEARDEN, University of Toronto, Toronto, Ontario. *Resupinate Thelephoraceae with amyloid spore walls.*—Bourdot and Galzin, in the Hyménomycètes de France, were the first to use the amyloid reaction of spore walls as a specific character in Thelephoraceae. In *Corticium* two species are segregated in a subgeneric section Amyloidea. Nine species of *Gloeocystidium* are included in a similar section. One species of *Peniophora* is listed as having amyloid spores. In these species the reaction in KII or Melzer's reagent is strong and spore walls become bright blue. The character is not mentioned in other genera though species of *Aleurodiscus* and *Vararia* (*Asterostromella*) and known to react similarly. A check of available species of *Corticium, Gloeocystidium* and *Peniophora* showed that in several other European species a positive but comparatively weak reaction resulted. When such species are included, together with a few North American forms, the number of species at present known to show an amyloid reaction is about 30. Other genera have not been consistently checked. The character of amyloid spores is not only a valuable aid in specific identification but gives promise of assisting in the problem of interspecific relationships. This character will also assist in the ultimate allocation of species to more natural genera in connection with the obvious necessity for generic revision in the family and particularly among that heterogeneous collection of resupinate forms now included in the artificially separated genera *Corticium, Gloeocystidium* and *Peniophora.*

SHARP, AARON J., University of Tennessee, Knoxville, Tenn. *Some fungi common to the highlands of Mexico and Guatemala and eastern United States.*—More than one third of a large collection of fungi from the highlands of Guatemala and Mexico have proven to be species which are also found in eastern United States. Included are species of *Amanita, Fomes, Irpex, Lactarius, Lenzites, Pleurotus, Polyporus, Schizophyllum, Trametes,* and *Xylaria. Amanita caesaraea* and *Lactarius indigo* are among the fungi sold for food in Puebla, Mexico.

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ERRATA

Page 141, the legend for the figures should read "Fig. 61–62.—Fig. 61 (right) . . . .—Fig. 62 (left) . . . ."

Page 230, column 1, line 17: "used" should be "tolerated."